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REDUCTION OF SILVER HALIDES AND THE MECHANISM OF PHOTOGRAPHIC DEVELOPMENT[1]

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Kodak Research Laboratories, Rochester, New York

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Exposure of photographic emulsions results in the formation of development centers consisting of latent-image silver; one development center is sufficient to produce the development of a whole silver bromide grain. The amount of latent image silver is determined by the amount of absorbed energy, but its effectiveness for development depends on its location and distribution, factors which are greatly influenced by preformed sensitivity centers. The number of quanta required for the developability of a grain is variable, but certain limits can be estimated.

Developers are reducing agents which react with exposed silver bromide at a much greater rate than with the unexposed material. A great number of reducing agents, inorganic as well as organic, possess this property. Hydroxylamine belongs to this group and its oxidation can easily be followed analytically; its rate of oxidation was investigated with silver ion in solution, with silver chloride sols, and with precipitated silver chloride. The catalytic influence of metallic silver was found in all cases; the reaction in solution proved that silver ions are adsorbed at the silver.

Some secondary features of development can be viewed in the light of the oxidation product theory, others in the light of the surface charge theory. The mechanism of development, however, cannot be explained by either of them. The development reaction can be regarded as belonging to a group of reactions which proceed with enhanced activity at an interface. It is discussed in connection with experimental data and theoretical considerations concerning this group.

I. DEVELOPABILITY OF EXPOSED EMULSIONS

Silver halides are not stable in the presence of a reducing agent, but react to form metallic silver. This property of the silver halides, although a necessary condition for photographic development, is not sufficient. A reducing agent cannot be a developer unless its reaction rate with exposed silver halide is of a higher order of magnitude than its reaction rate with the unexposed material. Only a very weak reducing agent will differentiate between exposed and unexposed pure precipitates of silver halide (79), but many developers can be found for silver halide sols. Even more reducing agents act as developers in photographic emulsions.

Photographic emulsions are not true emulsions as defined in colloid chemistry, but consist of silver halide grains of microscopic dimensions embedded in gelatin. In the negative material, the emulsion grains are silver bromide crystals, sometimes containing a small percentage of silver iodide. The diameter of these grains normally lies between 1 and 0.1 μ. In some positive emulsions silver chloride is used. In most emulsions, excess halide ions are adsorbed to the surface and, in the case of optically sensitized emulsions, dyes are adsorbed to the surface.

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[1] Communication No. 821 from the Kodak Research Laboratories.

After a photographic emulsion has been exposed to light which it can absorb, subsequent treatment with a developer produces silver in an amount which will be a measure of the exposure. In this way, a developed image of the photographed object is obtained. Even though the emulsion does not show any visible change after the usual exposure, it is safe to assume that hidden changes occur which give the shape of the image after development. The term "latent image", which was chosen for the effect of exposure on the photographic emulsion, is thus accounted for. The nature of the latent image has been the object of a long controversy between exponents of widely different opinions. This controversy may be regarded as closed, however, since the identification of the latent image with metallic silver is reasonably well supported by all the available evidence.

A. *Photolytic silver*

The production of silver by prolonged exposure to light had been established much earlier and was the subject of numerous studies. Eggert and Noddack (20, 21) found that the amount of photolytic silver followed Einstein's law of photochemical equivalence, so that the number of reduced silver atoms is equal to the number of quanta absorbed. This relation can be found only in the presence of a halogen acceptor. For moderate exposures gelatin is sufficient to prevent recombination, but for higher exposures it is necessary to add other acceptors, like sodium nitrite, in order to obtain a quantum efficiency of 1. Eggert and Noddack assumed that their results were valid also for the silver of the normal latent image; their conclusions are supported by the investigation of van Kreveld and Jürriens (55), who developed a very sensitive method of detecting the silver by its absorption in the red region of the spectrum. They showed that the production of silver is proportional to the exposure over a very wide range, extending to exposures which are in the region of the latent image. Moreover, on extrapolation, the straight line obtained by plotting the amount of silver against the exposure goes through the point of origin of the coördinates.

It has been shown that two emulsions of equal absorptive power contain, after equal exposure, equal amounts of latent-image silver. Their photographic sensitivity, however, may differ widely, which means that the amount of silver[2] produced by development will be completely different in the two emulsions. It is clear that the mere formation of photolytic silver is not a sufficient condition for developability.

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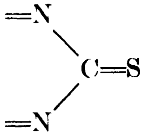
[2] The developed silver is usually measured by the optical density, as originally proposed by Hurter and Driffield (36). This density, the logarithm of the ratio between incident and transmitted light, was supposed to be proportional to the mass of the silver. Later investigations (31, 67, 78, 87, 103) showed that this relation does not always hold, but it is approximately valid over a considerable range if the degree of development is constant and only the exposure is varied.

B. Development centers and sensitivity centers

In their early investigations on photographic development, Sheppard and Mees (92) discovered the important part played by the individual grains. Microscopic investigations revealed that, if development is carried to completion, every grain which starts to develop is reduced throughout. Development in one grain does not affect the neighboring grains, however, unless they are in such close contact that "clumping" occurs (114). Large-sized grains can be expected to produce, therefore, a much greater density than grains of small size, since a much larger area is affected if development is started in a large grain. By microscopic observations on arrested development, Hodgson (34) was able to locate the starting points of development on the grains. He found that the formation of one center was sufficient for the development of the whole grain. The development centers subsequently were made the subject of numerous studies. Svedberg and Andersson (116a) developed a technique for investigating emulsion layers consisting of single-grain layers of one size-class, and Toy (118) succeeded in preparing single-grain layers not only of equal size but also of equal shape. It was always found that the centers were distributed at random over the various grains, the probability of center formation increasing with the projective area of the grains.

Thorough investigations of the effect of grain size on developability were carried out by Sheppard with Wightman and Trivelli (143 to 150). Within the same emulsion, the projective area of the grains was found to be the decisive factor for the formation of development centers, and an attempt was made to interpret the density curve on the basis of the different grain-size classes. However, the difference in the sensitivity of various emulsions could not be explained in the same way, and it became more and more evident in the course of the investigation that some inherent property of the emulsion played an even more important part.

In the subsequent search, it was found that this property was connected with the nature of the gelatin used in the after-ripening process (96). It was assumed that an impurity is responsible for the effect on sensitivity. This impurity, tentatively called gelatin X, was concentrated from the gelatin by various disintegrating operations, using the effect on sensitivity as an indicator. A comparison with the effect of plant seeds led to the discovery of the effectiveness of mustard oil and finally to the grouping of gelatin X with the thiocarbamides. The active group was supposed to have the constitution



which is able to form a complex with silver ion and finally to produce silver sulfide. Thiocarbamide was found to have, indeed, a strong sensitizing effect if it is used under conditions where silver sulfide can be formed. One part in 100,000 to 300,000 parts of silver bromide already gives the optimum effect; much more than this results in fog. This high efficiency is connected with the autocatalytic character of the thiocarbamide reaction, which results in the formation of concentrated centers of sensitivity (13). The silver sulfide specks on the surface of the grains are supposed to act as concentration centers for the latent-image silver and in this way to produce the development centers (101, 102).

Microscopic evidence for the concentration of the photolytic silver in discrete specks has been given by Trivelli and Sheppard (121), who concluded that the same process takes place in the region of much lower exposure, corresponding to latent-image production. This has recently been justified by means of the electron microscope, which gives results in lower exposure regions than the optical microscope. Figure 1 shows a photograph of the silver specks on a silver bromide grain, obtained by Hall and Schoen (30).

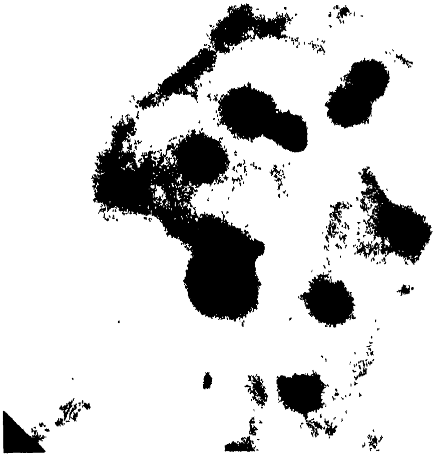


FIG. 1. Photolytic silver particles (Hall and Schoen)

The existence of sensitivity centers is supported by experimental evidence showing that the formation of discrete development centers is not necessarily connected with the action of light, but can also be produced by chemical agents, such as hydrogen peroxide, sodium hypophosphite, sodium arsenite, and others (11, 15, 16, 19, 97, 100, 116). Clark (17) found, further, that the sensitivity to arsenite could be destroyed by treatment with chromic acid in the same way as the sensitivity to light.

C. Formation of the development centers

A theoretical explanation for the action of the sensitivity centers is given by the Gurney–Mott (28) mechanism of latent-image formation. On the basis of this hypothesis it is possible to account not only for the primary act connected with the absorption of light, but also for the subsequent concentration of the reaction products.

The evidence for the primary act was furnished by the inner photoelectric effect. Vanselow and Sheppard (105, 108, 125) studied the instantaneous change of potential on illumination of silver halides, i.e., the photovoltaic effect; Toy (119) and Harrison (120) investigated the photoconductivity. Both phenomena signify the release of electrons by absorption of light, so that they can be shifted over some distance inside the crystal.

Webb (133) interpreted the internal photoelectric effect in the silver halides on the basis of the quantum-mechanics model of the structure of ionic crystals. According to this conception, the electronic levels of the individual lattice elements will be affected by the surrounding elements, so that each electronic level covers a variety of energies which are freely interchangeable between the elements of the same kind. Thus, the electronic levels will be represented by bands or zones instead of the sharp lines characteristic of the gaseous state. In silver bromide an electron in the lowest zone, corresponding to the ground state of the bromide ions, will have no freedom of movement, since all available positions for electrons are filled. A zone of a higher level, however, may have unfilled positions into which the electrons can move. Such a band is called a conduction band. The absorption act in the silver halide was assumed to consist in the elevation of an electron from the ground zone formed by the bromide ions to a conduction band formed by the silver ions where it could move through the crystal.

Gurney and Mott adopted this interpretation of the primary act (74) and supplemented it by a plausible explanation of the formation of centers. The electrons traveling through the crystal will eventually be trapped by some irregularity or impurity in the lattice. An electron trapped in this way would resist the trapping of another electron because of the electrostatic repulsion. This fact presented a serious difficulty to the theory of latent-image formation, until Gurney and Mott bridged the gap by taking account of a crystallographic discovery (51, 129). Silver bromide contains some silver ions which are not situated at the regular lattice positions, but at interstitial places, and the ionic conductivity of silver bromide can be attributed to these interstitial ions. Gurney and Mott pointed out that such interstitial ions can be expected to appear more frequently in the vicinity of an irregularity in the crystal, such as is produced by a silver sulfide speck. An effect of the sensitivity speck on the ions in its vicinity had, indeed, already been suggested in 1928 by Sheppard and Trivelli (103a). An electron trapped at this speck will give it a negative charge and attract an interstitial silver ion from the neighborhood, which then should join the speck and form a silver atom. This process of alternate electron-trapping and silver-ion attraction can be repeated, and the probability of growth should increase with the size of the speck. The part played by the sensitivity centers was very plausibly explained in this way.

The Gurney–Mott mechanism regards latent-image formation as consisting of two different processes: the electron movement and silver-ion migration. The existence of two components in latent-image formation had previously been suggested by Webb (131), because of the fact that reciprocity-law failure at high intensities and at low intensities depends on temperature in an entirely different way. The Gurney–Mott mechanism not only sheds light on the temperature dependence of reciprocity-law failure but explains the phenomenon itself.

The reciprocity law, formulated by Bunsen and Roscoe for photochemical reactions, postulates that the rate should be proportional to the product of time and intensity of exposure and independent of the value of either separately. This law has been recognized as a natural corollary of the law of photochemical equivalence, and both laws are supposed to be valid for any primary photochemical process. The experimental rate of a photochemical reaction, however, includes the effect of subsequent processes which may change the functional dependence altogether.

It was soon recognized that in photography the product of time and intensity is not sufficient to determine the effect of an exposure. This effect is relatively smaller at very high intensities as well as at low intensities. No satisfactory explanation of this failure of the photographic reaction to comply with the reciprocity law appeared until Gurney and Mott introduced the complex mechanism of center formation. The explanation of reciprocity failure at low intensities assumes a thermal activation of the spontaneous disintegration of centers below a certain size (135, 138). This effect is expected to occur at low intensities and high temperatures and to decrease and finally to disappear with the lowering of the temperature—a result established experimentally. At very high intensities the migration rate of the silver ions will play a decisive part, since it will not be high enough to permit neutralization of the electrons in the traps as quickly as they arrive. As the temperature is lowered, the migration rate of the silver ions will be lowered, while the electron migration will not be affected. Thus, with decreasing temperature the high-intensity reciprocity failure will become evident already at lower intensities. The effect will become more and more pronounced until, at very low temperatures, the migration of ions will become negligible, with the result that no photographic effect is produced. Ample experimental support for the theoretical interpretation of the high-intensity reciprocity failure was furnished by Webb and Evans (134) and by Berg and Mendelssohn (5, 6). The former authors demonstrated that exposure at low temperatures can be made efficient if it is applied in short flashes alternated with dark periods at room temperature. In this way, sufficient time is provided for the migration of silver ions.

D. Distribution of development centers

There are two possible sequences of a center disintegration: a true reversal or the formation of one or more new centers. The Herschel effect, consisting in the disintegration of centers by red or infrared radiation, results in a true reversal. Both developed silver and photolytic silver decrease in this case. Reciprocity-law failure, however, is not known to affect the amount of photolytic silver. This can be explained if new centers are formed which are not accessible to the developer.

A developer not containing a solvent for silver bromide will be able to start development only at latent-image centers situated at the surface of the grains. The developer solutions usually contain sodium sulfite, which has some solvent action on silver bromide, and consequently penetrate slightly deeper into the grain at the start of development. Centers in the interior of the grains, however, will still be inaccessible and therefore useless for the start of chemical development.

The existence of latent-image silver in the interior of the grains was suggested as early as 1894 by Kogelmann. Lüppo-Cramer (64) concluded that interior nuclei are present from the fact that treatment with iodide can restore developability which has been destroyed by treatment with chromic acid. He interpreted this as an exposure, by the solvent action of the iodide, of the interior nuclei, after the destruction of the external nuclei by chromic acid (18). This interpretation was supported, for concentrated iodide solution, by Sheppard, Wightman, and Trivelli (98, 99). Evidence for the relative distribution of centers between surface and interior, however, was collected only in recent years.

Meidinger (70) published extensive investigations on the distribution of photolytic silver. He found that increasing exposure favored the surface silver as against the silver in the interior. However, these results are not applicable to normal photographic conditions. The exposures needed for the analytical detection of photolytic silver are much greater than those used for photographic purposes. In addition, the silver bromide grains used in these investigations had several times the size of even the largest grains found in commercial emulsions, and had been prepared in the presence of inert gelatin. The same material was used by Kempf (52), who investigated the distribution of development centers at a single exposure level. The centers were found to be more numerous in the interior than at the surface. For very low exposures of photographic emulsions, however, Kornfeld (54) found the development centers to be almost entirely at the grain surface. Since at these low exposures the latent image can be expected to collect exclusively at the preformed sensitivity centers, this result indicates that nearly all the sensitivity centers are present at the surface of the grains. This, in turn, agrees with the fact that the production of sensitivity centers in after-ripening occurs when crystal growth is practically completed. With increasing exposure, new centers are created in the interior. At very high exposures, the ratio is reversed: practically all the centers are found in the interior. Berg, Marriage, and Stevens (7), in an extensive investigation on the dependence of surface centers and interior centers on temperature, intensity, and exposure time, established the fact that the density curve of the *interior centers* is less affected by temperature than that of the surface centers, that it shows no high-intensity reciprocity-law failure, and that the density increases in the solarization region. This latter result finally confirms the interpretation of solarization as a result of the rebromination of the grain surface (139) and agrees with the increase of photolytic silver in the region of solarization (2).

E. Limits of effectiveness for the centers

Various attempts have been made to determine the number of silver atoms necessary to create a development center. This number should not be expected to have a fixed value, since the effectiveness of a center will depend upon orientation, as was emphasized by Sheppard (106). An attempt can be made, however, to find the range of variation for the limiting number of silver atoms under varying conditions.

Reinders and Hamburger (84) and, later, Reinders and DeVries (85) deduced from their experiments the very small and constant limiting number of three to four or, respectively, four silver atoms. Their method was as follows: silver vapor was condensed on a glass plate in invisible minute quantities and then submitted to physical development. In this way, they determined the minimum amount which, after development, could produce one or more silver specks. For this minimum amount, the probability was calculated for the silver being distributed in groups of one, two, three, four, or more atoms. The number of silver specks found experimentally coincided with the calculated number of groups of four atoms. Apart from the fact, however, that these results apply only to physical development under conditions which differ from those for the development of photographic emulsions, it must be realized that the probability calculations rest upon the assumption that condensation of the silver atoms proceeds without any interaction between the groups. It seems rather doubtful that this assumption is justified, in view of possible adlineation.

The mathematical analysis of the characteristic curve (density plotted against logarithm of exposure) gave another method of approach to this problem. Svedberg (116, 116a) was the first to give a statistical evaluation of the surface area of the grains made just developable by a certain exposure. He showed that the dependence on the logarithm of the exposure found in the density curve could be approximated by this function. The assumptions necessary for this approximation were studied thoroughly by various authors, especially Silberstein and deLanghe. Silberstein finally came to the conclusion (111, 112) that the shape of the experimental curves indicated that only a very small part of the absorbed quanta was put to effective use in the grains. He estimated the number of quanta utilized as less than three per grain. It was pointed out by Webb (136, 137, 140), however, that there is an alternative interpretation of the density curve. The very small numbers of utilized quanta must be assumed if no variation of sensitivity exists between the grains. With a very wide range of sensitivity, however, some of the grains must be assumed to utilize several thousand quanta in order to fit a curve of the desired shape. Webb succeeded in reproducing the density curves of many commercial emulsions by his method. He showed (142) that the number of quanta utilized per grain assumed for the various grain-classes is of the same order of magnitude as the number of absorbed quanta per grain, as calculated from the experimental values of time and intensity of exposure, absorption coefficient, and size of grain.

In another attempt to get information on the number of quanta used in the formation of an effective development center, Webb (130, 132, 141) determined the critical frequency of intermittent exposure above which the developed density corresponds to the average intensity of the intermittent light. At low frequencies of intermittent exposure, the density corresponds to the intensity of the flash period instead. The critical frequency thus indicates the rate of intervals necessary to produce the same reciprocity-law failure as the corresponding continuous exposure, and thus gives the rate of quanta accepted by the most effective development center. The time of exposure then gives the number of quanta used for the formation of the development center. With the emulsions employed, some grains required at least sixty-four, and probably more than one hundred, quanta for latent-image formation.

By this method is obtained the number of quanta which actually takes part in the formation of a development center. However, possible losses by partial disintegration of the center make this number only an upper limit for the number of silver atoms added to a center during exposure. It could be taken as an upper limit for the total number of silver atoms forming a development center only in grains which do not contain preëxisting sensitivity centers.

As yet another method of approach, the number of silver atoms which must be produced in order to insure developability of the grain by x-ray exposures can be calculated. Glocker and others pointed out that in the photochemistry of x-rays the primary process of absorption does not play a dominant part, as it does in the photochemistry of ultraviolet and visible rays. The very large quantum releases a number of electrons which is proportional to its energy content. Each of these secondary electrons can start a chemical reaction, and the rate of this reaction is thus proportional to the energy content of the x-ray quantum, or inversely proportional to its wave length. For the photolytic silver in the photographic plate, this relation was confirmed by Günther and Tittel (27) between 0.245 Å. and 1.54 Å. The number of silver atoms per grain at 0.245 Å. was about one thousand. Assuming that the inverse proportionality with the wave length can be extrapolated, one should expect about *thirty-five silver atoms* to be released at 7 Å., and between *five* and *six* at 45 Å. These figures can be used in connection with the investigations by Broili and Kiessig (11), by Mülbach (77) and by Hirsh, Jr. (32, 33). Broili and Kiessig took the numerical (instead of the logarithmic) density curves of four emulsions. With x-rays these should be straight lines, indicating that every quantum absorbed makes a grain developable (26). Straight lines were, indeed, found with a radiation of 1.54 Å., but not with 45 Å., and Agfa Contrast emulsion even showed quite clearly a sensitivity threshold for the longer wave length. Mülbach, on the other hand, found straight lines for 4 Å. and 7 Å. with his emulsions, and no sign of reciprocity-law failure. Hirsh found an inflection point by exposing Eastman IV–0 plates to radiation of 14 Å. and even 11 Å. (His claim does not seem justified for 6 Å.) These results seem to show that, with the emulsions investigated, every grain is made developable if thirty-five silver atoms are produced, while twenty-two are not sufficient and five still less.

Again it should be emphasized that these figures cannot give upper limits for the number of silver atoms present in a development center, since the variation from emulsion to emulsion clearly indicates the participation of sensitivity centers with their unknown content of silver and silver sulfide. They could be regarded as lower limits only if the assumption were made that all the silver atoms formed by the x-ray quantum are collected in one center at the surface. In this connection, it ought to be mentioned that the unripened Lippmann emulsions do not give a straight-line curve, even with α-rays, as was shown by Blau and Wambacher (9). They calculated that α-rays should liberate more than two hundred silver atoms per grain along their path.

II. THE DEVELOPING AGENTS

The known developing agents are both large in number and diversified in chemical composition. The following outline is not intended as a comprehensive listing of the agents known to possess developing power, but rather as an indication of the variation in chemical types encountered among even the more common developing agents.

(*A*) *Inorganic:* (*1*) Metallic: various ferro- and molybdo-complex ions such as the oxalates and malonates, ammoniacal cuprous oxide, ferro-fluoride complex, and probably ferrous ion itself. (*2*) Non-metallic: hydroxylamine, hydrogen peroxide, and sodium hydrosulfite.

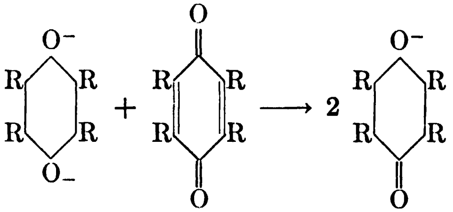
(*B*) *Organic:* (*1*) Aliphatic hydroxyketones: ascorbic acid (vitamin C). (*2*) Aliphatic aminoketones: 1-phenyl-3-methyl-4-amino-5-pyrazolone. (*3*) Aromatic compounds obeying the Andresen–Lumière rules, i.e., compounds possessing at least two hydroxyl or two amino groups, or one of each, in the ortho or para position on a single aromatic nucleus. The amino groups can be substituted, but the hydroxyl groups cannot. The number of such compounds is very large. Included are such relatively simple representatives as hydroquinone, *p*-aminophenol and *p*-phenylenediamine, and such complex structures as hematoxylin and brasilin. The number of active groups may at times extend to the limit. Thus, 1,2,4,5-tetrahydroxy-3,6-diaminobenzene is a usable developer in somewhat acid solution. Not all compounds conforming to the Andresen–Lumière rules are developers, as, for example, dicyanohydroquinone. (*4*) A very limited number of aromatic compounds containing hydroxyl or hydroxyl and amino groups in the meta positions are usable developers, e.g., trioxymesitylene. (*5*) Some aromatic compounds containing not more than one hydroxyl or amino group on any nucleus are developers in sufficiently alkaline solution, e.g., hydrocoerulignone and 1-naphthol.

The developing agents must be capable of reducing silver halides to metallic silver, but this is not a sufficient condition. It was pointed out at the beginning of this article that the developing agent must reduce the exposed silver halide grains at a substantially greater rate than the unexposed grains. Some reducing agents, such as sodium stannite, do not fulfill this condition and therefore are not developing agents.

The theory of the development process is concerned essentially with the reason for the selective reduction of the exposed grains. Before considering the reaction of the developer with the complex photographic emulsion, however, it is desirable to treat the reactions of the developing agents under less complicated initial conditions.

A. Oxygen oxidation

Most of the organic developing agents belong to types which can form semiquinones as oxidation intermediates (71, 72). Durohydroquinone, for example, is oxidized to duroquinone by way of the radical durosemiquinone (73). The percentage of the radical in equilibrium with the fully oxidized and fully reduced forms increases markedly with the alkalinity of the solution. At pH = 8.71 the concentration of durosemiquinone in a solution of partially oxidized durohydroquinone is too small to be detected potentiometrically. However, it has an important bearing on the mechanism of oxygen oxidation of the durohydroquinone in this pH region, since the reaction is markedly catalyzed by the presence of the quinone. The kinetics of this catalytic process suggest that the oxygen mainly attacks the semiquinone which is formed by the interaction of the durohydroquinone and duroquinone (38). The rate of this reaction is proportional to the concentrations of duroquinone and divalent durohydroquinonate ion and is independent of the oxygen concentration. The rate-controlling reaction, therefore, appears to be:



The kinetics of the catalyzed oxygen oxidation of trimethylhydroquinone (ψ-cumohydroquinone) are identical with those of durohydroquinone at very low ψ-cumoquinone concentrations. With increasing concentration of the quinone, the reaction rate in this case becomes increasingly independent of it, and, at the same time, a dependence upon the oxygen concentration appears. In order to explain these results, Kornfeld and Weissberger (53) assumed the interaction of the semiquinone not only with oxygen but with the quinone as well.

The oxygen oxidation of the lower methylated hydroquinones and of hydroquinone itself is independent of added quinone, and the rate varies as the first power of the oxygen concentration (39). One is tempted to assume, for these reactions, that the region in which the reaction rate becomes independent of the quinone concentration is reached at very small concentrations. Qualitatively, the inhibitory action of sodium sulfite and certain thiol compounds could then be explained by their power to react with quinone, thus reducing the concentration of the latter below the critical value for maximum catalytic action. However, the fact that a linear relationship exists between the reaction rate and the reciprocal of the sulfite concentration does not agree with this simple explanation (40).

It appears quite possible that a chain mechanism of some sort is operative in the oxygen oxidation of hydroquinone and at least its lower homologues. The radical HO_2, which was assumed to play no kinetically significant rôle in the simple quinone catalysis mechanism proposed, may actually be of importance as a chain-carrying intermediate. Gee has used this radical in the mechanism which he suggests for the oxidation of hydroquinone (25), but his mechanism does not include the important rôle of the semiquinone. Chain reactions in similar oxidations have been proposed by Branch and Joslyn (catechol) (10) and by Haber and Willstätter (enzyme-catalyzed oxidation of pyrogallol) (29), but neither mechanism is satisfactory for the oxidation of the hydroquinone series.

B. Reduction of silver salts

There is evidence for a quinone catalysis in the reduction of silver ions by hydroquinone and durohydroquinone (49). A catalysis by the silver formed in the process is, however, far more important.

A pronounced silver catalysis is observed in the reduction of silver ions by hydroquinone, both in acid and in slightly alkaline solution (41, 49, 59). In the slightly acid region, where the catalyzed reaction is most amenable to quantitative study, the dependence of rate upon hydroxyl-ion concentration indicates that both non-ionized hydroquinone and the univalent ion take part in the reaction (pH range 5.15 to 6.27). The rate varies as the first power of the hydroquinone concentration and as about the two-thirds power of the silver-ion concentration. The latter fact suggests adsorption of the silver ions to the metallic silver. Direct experimental confirmation of such adsorption is obtainable from other sources (22, 81, 126). Veselovsky, who was careful to work with oxide-free silver surfaces, found that adsorption begins at a silver-ion concentration of 1×10^{-13}. In the presence of oxide, the null point occurs at a much greater silver-ion concentration, but in the silver catalysis considered here a freshly reduced silver surface is assured.

The kinetics of the reduction of silver ions by hydroquinone supply no evidence for the adsorption of hydroquinone to silver. Direct experimental determinations show that such adsorption, if it occurs at all, is very small (109). Earlier work by Rabinovitch and Peissachovitch (82) must be discounted because of errors in technique. Their procedure was to mix a silver sol with hydroquinone and to determine the concentration of the latter before and after ultrafiltration. Actually, much of the original hydroquinone used escaped analytical detection even before ultrafiltration, owing to oxidation, and no adequate correction was made for oxidation occurring during the ultrafiltration. In the procedure employed by Perry, Ballard, and Sheppard, oxygen and free silver ion were eliminated as far as possible, and all but 0.44 per cent of the hydroquinone used initially was accounted for in the filtrate (pH = 9.0). This they consider to be within experimental error.

The reduction of silver ions by hydroquinone has also been studied in the pH range 8.6 to 9.0, using the soluble sulfite complex as a source of silver ions. The reaction mechanism indicated is the same as in the lower pH region, except that an additional complication arises as a result of quinone catalysis. Principally, the univalent hydroquinone ion is involved in the silver-catalyzed reaction, but the quinone catalysis very probably involves the divalent ion.

A detailed study of the kinetics of reduction of pure silver halide by hydroquinone should prove of great value to the understanding of photographic development. The experimental difficulties, however, are great. An elaborate attempt to carry out such a program was made by Luther and Leubner (65), but the results were largely disappointing. They employed freshly precipitated silver bromide containing an excess of potassium bromide amounting to 0.0247 mole per liter. The work was well planned, but an adequate experimental procedure was lacking, and little of value can be deduced from the scanty data which they were able to accumulate.

Hydroxylamine is by far the most useful developing agent for a general kinetic study of the reduction of silver salts. This agent has the advantage that, over a fairly wide range of experimental conditions, it reacts almost quantitatively to give silver and nitrogen as reaction products. The nitrogen can be readily determined volumetrically without interfering with the reaction.

The reduction of silver nitrate in solution has been studied by this method over the pH range 3.7 to 4.45 (42). Reaction is detected only after a relatively long induction period. Beyond this, an S-shaped reaction curve is obtained. The induction period is considerably shortened by the addition to the reaction mixture of a nuclear gold sol, and results can be satisfactorily duplicated. The reaction curve is the same, whether it is based on measurement of nitrogen or on direct determination of silver (figure 2).

Addition of a silver sol to the reaction mixture at the start practically eliminates the induction period (i.e., the period in which no measured reduction occurs). The rate of the silver-catalyzed reduction varies approximately as the two-thirds power of the silver-ion concentration, in precise agreement with the relation found for the hydroquinone reaction. The dependence of the rate upon the hydroxylamine concentration decreased as the pH increased and also as the hydroxylamine concentration increased. The dependence of the rate upon pH indicated clearly that the ion, NH_2O^-, is the active species, and the variation of rate with concentration of this ion indicates adsorption of the ion to the silver. This latter is in contrast to the hydroquinone mechanism.

Kinetic studies on the reduction of the soluble silver sulfite complex at pH = 8.54 show that the mechanism is essentially the same as for silver nitrate. The undissociated complex itself is not attacked to any detectable extent. The silver-ion concentration supplied by the complex is of the order of 10^{-9}, which is comparable with that which silver chloride could supply during development in the presence of 0.1 *M* excess chloride.

The reduction of silver chloride, precipitated in the presence of excess chloride ion, yielded the typical S-shaped reaction curve in the region studied (pH 7.0 to 7.5) (45, 46). The initial reaction rate varied directly with the hydroxyl-

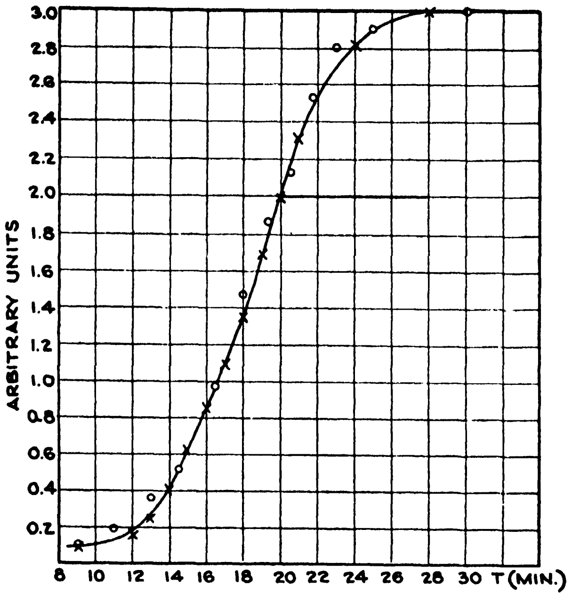


FIG. 2. Reduction of silver ions by hydroxylamine: ○, silver; ×, N_2 volume

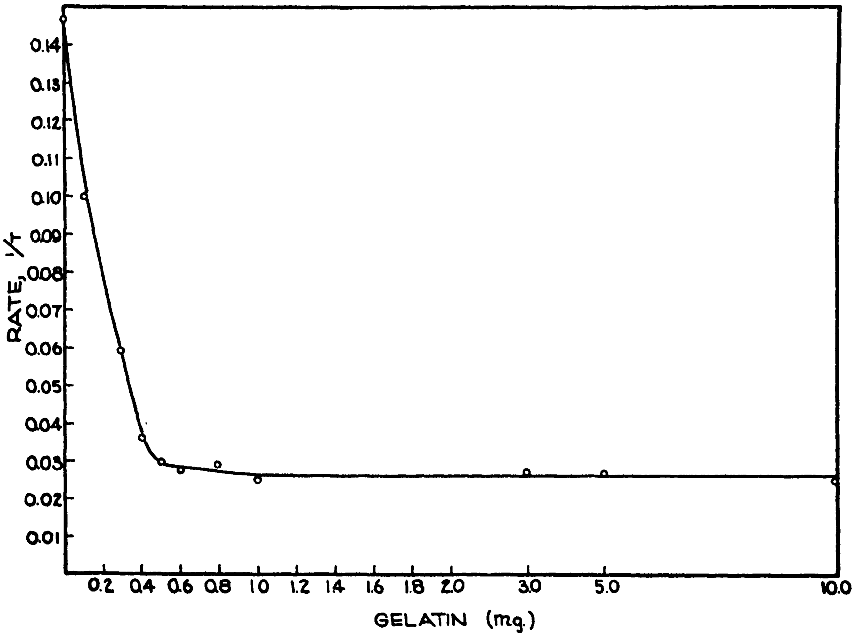


FIG. 3. Variation of initial rate with amount of gelatin

amine concentration and inversely with the chloride-ion concentration when the latter was relatively large. The rate varied approximately with the amount of available surface, and this relation held whether the surface was changed by aging or by partial coverage with a strongly adsorbed cyanine dye, such as 3,3′-diethyl-9-methylthiacarbocyanine. Addition of gelatin to the precipitate caused the reaction rate to drop sharply as the amount of gelatin increased, until a minimum rate was attained (figure 3). Further addition of gelatin had no measurable effect. Evidence from other sources shows that gelatin is rather strongly adsorbed to the halide (107), but probably not so strongly as the cyanine dye just mentioned.

The effects of gelatin and of the cyanine dyes supply important evidence concerning the site of the reaction. The dyes exert scarcely any effect upon the reduction of silver ions from sulfite solution at the same pH. Gelatin decreases the rate of the latter reaction, but the relative effect is much smaller than in the case of silver chloride, and a minimum rate is not attained even at fairly high gelatin concentrations. Indeed, the entire kinetic aspect is different in the reduction of silver ions from solution, showing that the attack on the silver chloride occurs on the solid phase. The fact that the reaction rate varies directly with the amount of available surface as more and more of the surface is covered by the dye suggests that the dye is uniformly and non-selectively adsorbed to the surface. This is in contrast to the reduction of mercurous chloride, where the greatest effect is observed during coverage of the first 10 per cent of the surface by the dye (50), a result which suggests that the active reaction centers selectively adsorb the dye.

Exposure of the silver chloride precipitate to white light, or nucleation by other means, largely eliminates the induction period. This effect, together with the regression which occurs when sufficient time elapses between exposure and reduction, is illustrated in figure 4. The rate of reduction of the nucleated precipitate varies with the 0.8 power of the hydroxylamine concentration. The temperature coefficient of the catalyzed reaction is substantially smaller than that of the initial reaction in the unnucleated precipitates. This is shown by the data in table 1. In this table, R_i is the rate of the initial reaction of the pure precipitate in terms of the reciprocal of the induction period; R_c is a measure of the rate of the silver-catalyzed reaction beyond the induction period; and R is the rate of the reaction in the nucleated precipitates in terms of cubic centimeters of nitrogen per minute. All values represent the average of triplicate runs.

The reduction of silver salts by hydrazine presents some points of similarity to the action of hydroxylamine, but also some important points of difference. The reduction of the soluble silver sulfite complex (47) and of the soluble ammonia complex (37) are both strongly catalyzed by colloidal silver. The reduction of silver chloride by hydrazine shows a definite induction period which, in some cases, is relatively large. However, in contrast to the hydroxylamine reduction, exposure of the precipitate to white light has only a slight effect upon the induction period or upon the subsequent course of the reaction. Nucleation of the precipitate by the action of hydroxylamine decreases the induction period without eliminating it and produces little or no effect upon the subsequent course of the reaction. Addition of the dye 3,3′-diethyl-9-methylthiacarbocyanine produces no effect until the surface of the precipitate is more than half covered. Further increase in the amount of dye added produces an irregular decrease in the reaction rate which is completely atypical of the hydroxylamine reaction. Gelatin decreases the reaction rate, but to a smaller extent than in the case of hydroxylamine, and a minimum rate is not attained. The data point to a mechanism involving the catalyzed reduction of silver

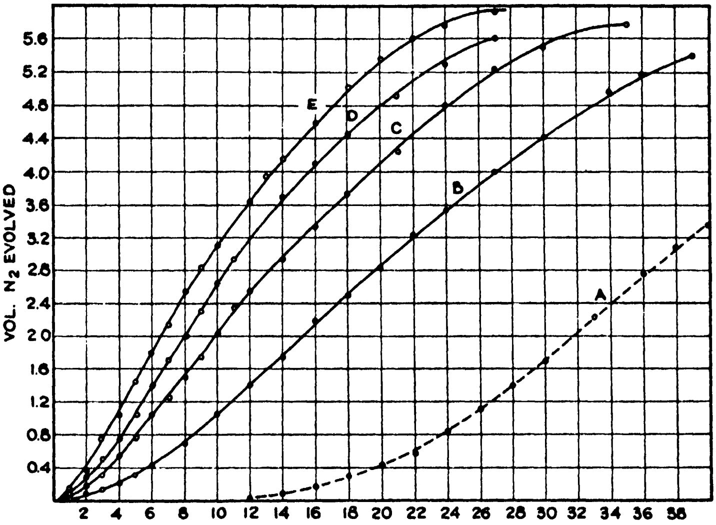


FIG. 4. Effect of age of exposure on reduction of silver chloride by hydroxylamine. Curve A, no exposure; curve B, exposure 17 hr. old; curve C, exposure 6 hr. old; curve D, exposure 2 hr. old; curve E, exposure 20 min. old.

TABLE 1

Temperature coefficients of reaction

TEMPERATURE	PURE PRECIPITATES				NUCLEATED PRECIPITATES			
					By NH_2OH		By light	
	R_i	Temperature coefficient	R_c	Temperature coefficient	R	Temperature coefficient	R	Temperature coefficient
°C.								
12.00	0.022		0.0090		0.058		0.145	
		4.46		3.35		3.25		2.80
20.03	0.073		0.0237		0.149		0.332	
		3.78		3.15		3.12		2.87
29.80	0.27		0.073		0.454		0.925	

ions from solution, a conclusion which is further supported by the effect of excess chloride ion upon the reaction rate.

The essential difference between the reduction of silver chloride by hydroxylamine and by hydrazine thus appears to be the following: reaction by the former agent involves direct attack upon the solid; reaction by the latter agent involves a preliminary solution of the silver chloride. Hydrazine is known to form complexes with the silver halides which are similar to the ammonia complexes, except that they are much less stable and decompose into nitrogen and silver at moderate temperatures (24). It is quite possible that the solvent action of the hydrazine plays an important part in determining the difference in action between this agent and hydroxylamine.

The comparative behavior of hydroxylamine and hydrazine in the reduction of silver chloride becomes quite suggestive when it is remembered that hydroxylamine is a rather clean-working developing agent, yielding good image development and reasonably low fog, whereas hydrazine yields an uneven image development and high fog. We can proceed a step further in comparisons and consider the action of sodium sulfite. This compound reduces silver ions, and the reaction is markedly catalyzed by colloidal silver, but the compound has a strong solvent action on silver chloride and bromide and it fails completely to develop an image. The silver catalysis is clearly in evidence in the reaction which occurs in a solution of the complex containing an excess of sulfite (48, 94) and the kinetics show that the silver ion, rather than the undissociated complex, is involved. Silver catalysis has also been observed in the reduction of silver bromide by sodium sulfite (13). The rate of even the catalyzed reaction is quite small at room temperature, however. The temperatures employed in the studies mentioned ranged from 50°C. to 88°C. Normal photographic emulsions become "fixed out" in the sulfite solution before much reduction is in evidence at room temperature. At higher temperatures, the silver formed is largely colloidally dispersed in the solution, and no image development is obtained.

Not all reductions of silver salts are characterized by autocatalysis. Fischbeck and Schnaidt (23) found that the reduction of single crystals of silver chloride by gaseous hydrogen proceeded without an induction period and without influence by reaction products. The reaction velocity was proportional to the silver chloride surface. The heat of reaction is about 9.6 kcal. The activation energy obtained for the reduction of silver chloride solidified from a melt was 61.0 kcal., numerically equal to the heat of dissociation of the salt (61.2 kcal.). However, precipitated silver chloride yielded the much lower value of 20.7 kcal.

Furoin and desylamine reduce silver ions in solution without evidence of silver catalysis (48). The reaction here appears to involve the enolization of the reducing agent as the rate-controlling process. The reaction is largely independent of the silver-ion concentration, and the rate is the same as that of reaction with oxygen. Sodium stannite is a very vigorous reducing agent, and the rate of reduction of most silver salts by this agent is very high. The only reaction of this agent in which silver catalysis has been detected is the reduction of the very slightly dissociated soluble silver iodide complex, where reaction probably involves the complex itself and not the silver ions. No silver catalysis is observed in the reduction of the silver thiosulfate complex, where reaction definitely involves the undissociated complex ion.

C. Development of the photographic emulsion

Two methods of approach which have been applied with some success to the solution of the mechanism of development in the photographic emulsion itself depend upon microscopic observations of the partially developed or developing grains and upon a study of the over-all kinetics of the process. We shall consider the results obtained by each method in turn.

It was pointed out in section I that development starts from latent-image centers and proceeds until eventually the entire grain is reduced. A hydroquinone developer containing only a small amount of sodium sulfite will effect the reduction of a pure silver bromide grain in a quite orderly fashion so far as the optical microscope reveals, and the silver particle remaining at the end of the process is a fairly good pseudomorph of the original grain (62). On the other hand, some of the more energetic developers such as Elon produce considerable distortion in the shape of the grain. During the development process, the grain as a whole appears to be constantly vibrating, and threadlike protuberances are thrown out. The mass of silver remaining at the end of the reduction bears little resemblance to the original (123).

Scheffer, in one of the earliest microscopic investigations of partially developed grains, observed the presence of fine filaments of silver jutting out from the surface of the grain (86). Some were so fine as to be barely visible at 2000 × magnification. Subsequent workers failed to obtain the striking display of fine filaments which Scheffer observed. The photolytic reaction proceeding under the influence of the very intense radiation applied has to be considered in his case. However, a formally similar phenomenon has recently been observed in photographs taken through the electron microscope.

Hall and Schoen (30) observed a seaweedlike mass of filaments formed when silver bromide grains were developed on the electron microscope object holder (20,000 to 50,000 × magnification). Grains developed on a photographic plate were not the same in general appearance, but, when completely developed, showed a filamentary structure only around the edges. The major portion of the grain was completely opaque. Similar observations were made by von Ardenne (1). Figure 5 shows an electron micrograph of a silver bromide crystal which was developed for a very short time, after which the unreduced silver bromide was dissolved out in sodium thiosulfate solution. A faint outline can be seen in the background where the original crystal has left its trace in the gelatin. A number of dots can be observed where development has just started. In the larger areas of silver, development has proceeded much farther and has extended rather irregularly over the crystal surface.

The filament phenomenon is not observed with physical development in which the grain is first dissolved and the latent image then developed by means of a silver-producing solution (silver salt and suitable reducing agent). The reduced silver in this case is observed only in the form of regular clumps.

Kinetic studies on the reduction of individual silver halide grains are difficult experimentally, and the data available from direct observation are quite limited. Steifon has made a series of photomicrographs of the progress of development by hydroquinone of very large (6 to 8 microns) insensitive silver bromide grains, and from these has determined the rate of reduction of the individual grain (83). In these photographs, the silver area spread over the surface of the grain in a regular fashion, and the length of the radius of the silver area increased linearly with the time of development. Thus, the rate of reaction was proportional to the visible interface between silver and silver bromide.

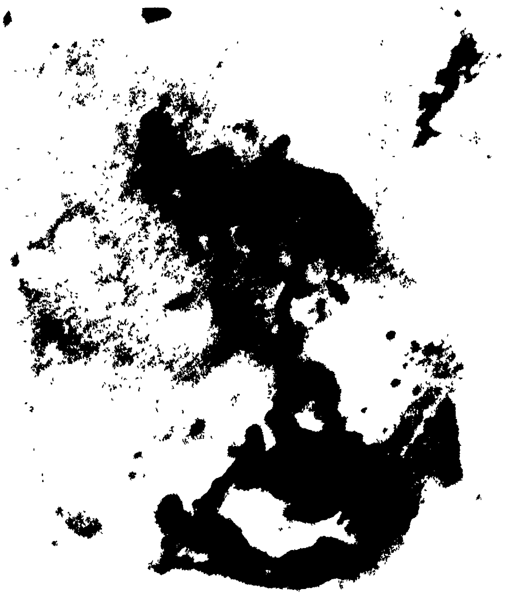


FIG. 5. Partially developed silver bromide crystal. Undeveloped portion dissolved out (Hall and Schoen).

Previously, Meidinger had made microscopic observations on the development of large silver bromide grains prepared in the same way as those employed by Steifon. Meidinger used a heavily bromided Elon hydroquinone developer, and collected the following data: (*1*) the number of grains which had begun to develop after a determined time; (*2*) the number of completely developed grains; (*3*) the mean time elapsing between the first trace of development detected under the microscope and the complete reduction of the grain (68, 69). The time elapsing between the beginning of penetration of the developer into the layer and the first detected trace of development (the initiation period) decreased with increasing illumination until the solarization region was reached. The rate of development beyond the initiation period, however, was independent of the amount of exposure. At the lower bromide concentrations employed, the rate of initiation decreased much more sharply with increasing bromide-ion concentration than the rate of development beyond the initiation period. Meidinger concludes that the velocity of development is made up of two components: The first depends essentially upon the probability of initiation of development in the crystal, and is dependent upon the exposure. The second is the rate of reduction of the crystal once reaction starts and is independent of the exposure.

It is unnecessary to assume that Meidinger's two components require two distinct reaction mechanisms. The observed dependence of the initiation period upon exposure and bromide-ion concentration are well accounted for by an effect which the electric charge on the silver bromide grain has upon the rate of development. This effect is particularly evident in the early stage of the reaction.

The lower portion of the density time of development curve will be concave, approximately straight, or convex, depending upon several factors. Experiments covering the action of fourteen developing agents belonging to several chemical types and employed under simplified conditions showed that a close correlation exists between the shape of the lower portion of the curve and the *charge on the effective developing agent* (43). The charge on the agents employed varied from 0 for certain members of the *p*-phenylenediamine group to −3 for sodium hydroquinonemonosulfonate. Each charge-group yielded its own particular type of curve (figure 6), irrespective of considerable diversity in the chemical nature of the constituents.

Certain surface-active agents which reduce the effective charge on the silver halide grains, such as phenosafranine and thallous ion, markedly increase the rate of development by members of group −2 or −3, but have only a slight effect upon development by members of group −1 and none whatsoever upon group 0. A similar observation applies to Luppo-Cramer's neutral-salt effect (63).

The charge effect has been explained as follows: The repelling force exerted upon an approaching developer ion by the negative charge of the adsorbed halide layer will be proportional to the charge of the ion itself. Only a fraction of the negatively charged ions will possess sufficient kinetic energy to overcome this repulsion, and the percentage will decrease rapidly as the charge increases. Those ions which, by virtue of their excess kinetic energy, approach closely enough to the latent-image centers and which, in addition, possess sufficient activation energy will react to produce metallic silver. As the silver speck grows in size, the protective layer becomes more and more disrupted in this region. The resulting decrease in charge permits an increase in the effective concentration of the developing agent at the reaction zone, and hence an increase in the reaction rate. The greater the charge of the developing ion, the greater will be the relative increase.

The effect described will be pronounced only in the early stages of development of the individual silver halide grains, and will probably disappear before the silver speck becomes very large. As expected, increase in the concentration of excess bromide ion has a greater effect upon the induction period of the charged developers than upon the subsequent reduction rate, in line with Meidinger's observations, and the greater the charge on the developing agent, the greater is the relative effect upon the induction period.

The autoaccelerating effect just described is quite distinct from the catalytic autoaccelerating action of silver produced during development. The effect of the increased silver interface becomes evident in development by such uncharged agents as diaminodurene, if the amount of silver formed is plotted against the time of development. Generally, the optical density is taken as a measure of the amount of silver, with the implication of a linear dependence. This proportionality, however, does not always hold (*cf.* footnote 2). Under the conditions employed in the preceding work on the shape of the development curve, the relationship

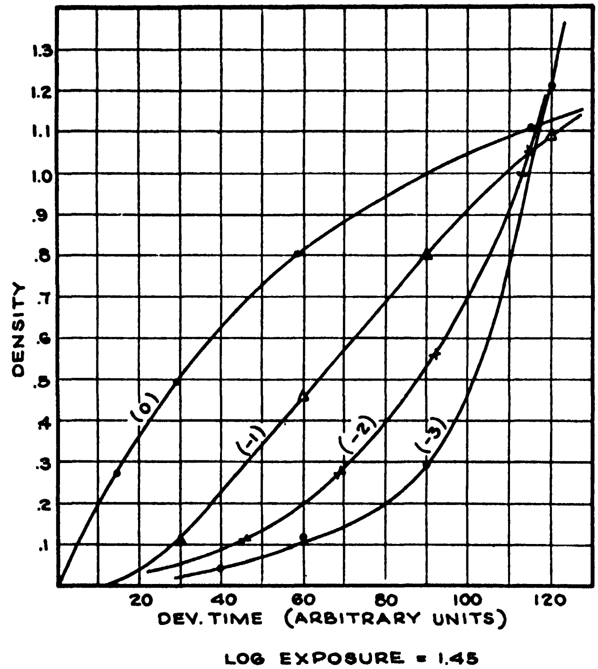


FIG. 6. Effect of charge of reducing agent upon shape of development curve. ×, hydroquinone; △, *p*-aminophenol; ●, diaminodurene; ○, sodium hydroquinonemonosulfonate.

$$\log \text{Ag} = 1.5 \log D + \text{Constant}$$

held good to fair approximation for constant exposure. When density is plotted against time, this non-linear relation between silver and density obscures the increase in reaction rate with increasing size of the silver-silver halide interface during the early stages of development.

Kinetic studies of the development of a simple commercial emulsion by hydroquinone have been carried out under specially simplified development conditions (44). The emulsion was a thin, unsensitized, pure bromide material of fairly uniform grain size. The developer solutions contained only the reducing agent and buffer and were used in the absence of oxygen. In the pH range employed (8.0 to 8.9) the development rate was sufficiently low to minimize diffusion effects. Analysis of the data showed that the rate of development throughout the entire course varied quite accurately as the 0.55 power of the concentration of the divalent ion $C_6H_4O_2^{--}$. The same variation was obtained when small amounts of sodium sulfite were added to the hydroquinone solution, even though the sulfite reduced the actual rate of development severalfold. The oxidation product, quinone, when added to the sulfite-free developer at the start of the reaction produced an increase in rate, supporting Staude's earlier observation of the accelerating effect of oxidation products (113, 114). The probable reason for the retarding effect of sulfite lies in its ability to react rapidly with quinone, forming the soluble hydroquinonemonosulfonate.

The unique dependence of the development rate upon the concentration of the divalent ion, rather than upon the non-ionized hydroquinone or the univalent ion, supports the suggestion of Sheppard and Mees that the active developing agent is the divalent ion (93). The occurrence of this ion to the one-half power in the kinetic expression is evidence that the ion becomes adsorbed prior to reaction. The site of the adsorption must be deduced from other evidence. Rabinovitch has suggested that the adsorption is to the silver, but the experimental evidence available does not support this assumption (109). A direct investigation of adsorption to silver bromide in alkaline solution is not feasible because of the chemical reaction which occurs. Wulff and Seidl, however, demonstrated that resorcinol is adsorbed from alkaline solution by silver bromide (152), and argued by analogy that hydroquinone likewise would be adsorbed. Sheppard and Meyer had suggested earlier that the first step in development is the adsorption of the reducing agent to the silver halide, forming a complex between the silver ions and the developing agent (95).

The importance of the accelerating action of oxidation products in the development process has been strongly emphasized by Staude and other workers in Luther's laboratory. Staude has even suggested that development would not take place at all in the complete absence of the oxidation product, and states that a reducing agent is a photographic developer "if it can form with its next oxidation product an addition compound which needs to be stable only in the solid phase or adsorbed to an interface, respectively" (115). However, the maximum accelerating effect which Staude observed was obtained with hydroquinone and amounted to only sixfold. In some cases, such as development by Elon or *p*-aminophenol, the oxidation product produces an acceleration only at the very start of development and retards the subsequent reduction (43). No acceleration at all has been observed with the *p*-phenylenediamine developers. On the contrary, agents which combine with the oxidation products in this case markedly increase the rate of development throughout the entire range. Staude's generalized definition obviously fails to account for this fact and for the developing power of hydroxylamine, whose oxidation product is nitrogen. Furthermore, addition products of the type he suggests are very improbable in a number of other cases, such as those of ferro-oxalate, ascorbic acid, and diaminodurene.

III. THE MECHANISM OF DEVELOPMENT

There has been a tendency in photographic theory to treat development as a unique phenomenon. Actually, there is ample evidence to link development to a whole series of reactions which proceed at an interface between reactant and product. The thermal decomposition of silver oxalate and the reduction of cupric oxide by hydrogen are well-known examples of such reactions. The general significance of the interface was first recognized by Langmuir (58).

The original explanation of the action of photographic developers is the classic Ostwald–Abegg supersaturation mechanism, which is now of no more than historical importance. A development hypothesis centered upon one particular aspect of the process, the electric charge effect, was proposed by Schwarz and Urbach. The excess halide ions adsorbed to the grains in the normal emulsion form a protective layer which will resist the approach of negatively charged developer ions. Schwarz and Urbach assumed that absorption of light by the silver halide grains resulted in the ejection of electrons from the surface halide ions into the interior of the grain, where they were caught by silver ions (89, 90, 91). The latent image was in this way identified with a loss of negative charge, regardless of the formation of silver specks. This extreme position was later abandoned, and the silver specks at the surface were regarded as responsible for the break or weakening of the protective charge (57). This hypothesis was employed with considerable success to explain certain development effects, such as the accelerating action of a few surface-active agents and the neutral-salt effect (60, 61), although some strongly adsorbed dyes, such as erythrosine and pinaflavol, showed the opposite of the effect expected (124).

As a general explanation of development, however, the surface charge mechanism cannot be accepted. If the selective rate between exposed and unexposed grains results from the charge effect, the indiscriminate reduction of silver bromide grains by the negatively charged stannite ion remains unexplained. Equally unexplained is the fact that certain acid emulsions containing excess silver ion, rather than halide, may be developed by conventional agents (12). If a negative charge layer exists at all in this case, which is doubtful, it must be very weak and could not afford the required protection. The most serious objection to the proposed mechanism is the existence of a whole series of developing agents, the substituted *p*-phenylenediamines, which must be considered to act as neutral molecules. Acid ionization of aromatic amines, when it has been measured at all, is too slight to be of kinetic significance. For example, the dissociation constants indicated for aniline and *p*-toluidine are of the order of 10^{-27} (66). The rate of development by the substituted *p*-phenylenediamines increases with increasing pH, but not in the manner expected for an ionization effect. Thus, the rate of development by diaminodurene is scarcely doubled by an increase in pH from 9 to 11. Many of these agents are good developers, and electron micrographs of the developed grains reveal the same general structure as that obtained with the charged developers. Physical development, which is so prominent in the action of *p*-phenylenediamine itself, does not play a significant part with these substituted agents.

The experimental data on the photographic induction period in development, discussed in a preceding section, enables us to consider the rôle of the protective charge layer in its true perspective. The effect of the charge upon the kinetics of the reduction is apparently a purely secondary one, in contrast to the primary catalytic effect of the silver nuclei. The charge effect can be detected with the singly charged developing agents, but it only becomes of real significance as a contributory cause of the difference in the rate of reduction of exposed and unexposed grains when the charge is 2 or greater. However, a double negative charge does not guarantee developing action, for stannite ion is completely nonselective.

Another special aspect of development was generalized by Staude, who assumed that the oxidation products of the developing agents were responsible for the developing action. This hypothesis, which is quite inadequate, was considered in the preceding section.

Still another special hypothesis was proposed by Gurney and Mott, in an attempt to apply their well-supported mechanism of latent-image formation to the development reaction (28, 75, 76). According to this mechanism, "the molecules of the developer hand over electrons to the metallic silver speck, and raise its potential relative to that of the halide grain. The interstitial silver ions which are present in the halide are then attracted to the silver speck; they will move up to it and adhere to it. Of course, as fast as the (interstitial) silver ions in the grain are used up, new ones are formed. The halogen escapes from the surface of the crystal, and not from the metal-compound interface. Interstitial ions, which may have come from quite distant parts of the crystal, flow through the crystal and join the metal at the interface."

If true, this extension would give a beautifully unified theory of latent-image formation and development. Unfortunately, there are serious objections. Inherent in the theory itself is the difficulty of providing a mechanism whereby the excess bromide ions can escape from the crystal. There is no experimental evidence that bromide ions can migrate through the crystal even at temperatures considerably higher than those normally employed in development. Theoretical calculations (35) show that the activation energy required for the migration of a bromide ion is at least five times greater than that required for the silver ion, and the activation energy of formation of the interstitial bromide ion is more than three times greater than that of the silver ion.

Moreover, the expected correlation between conductivity and development kinetics is completely lacking. According to this hypothesis, the rate of development should be a simple function of the number and mobility of the interstitial silver ions. Experimentally, this is not the case. The concentration of interstitial silver ions decreases in the series AgI > AgBr > AgCl, yet the development rate generally increases in this series.

The amount of current passing through the silver bromide crystal is proportional to the applied potential. However, no simple relationship exists between the rate of development and the redox potential of the developer. Even among the iron developers, where the redox potentials are well defined, it is observed, for example, that a ferro-citrate solution develops at a much smaller rate than a ferro-malonate solution of identical redox potential (8, 110). With hydroquinone, an increase in ΔE may even produce a decrease in the development rate (44).

Furthermore, the temperature coefficient of development is a function of the developer, not the silver halide, and the coefficient is usually much smaller than that of electrical conductivity. Dyes adsorbed to the silver halide surface should not affect the mobility of internal interstitial silver ions, yet they may reduce the rate of development by 100- to 1000-fold. Finally, it has been shown that the kinetics of reduction of mercurous chloride are formally very similar to those of silver chloride, although the Gurney–Mott mechanism is impossible in the former case (50).

Piper, in 1908, suggested (80) that development is an "action of a catalytic nature, the latent image being the catalyzer." Sheppard (94) pointed out that colloidal silver markedly accelerates the formation of silver in a silver nitrate–sodium sulfite solution and suggested that the latent-image silver facilitates the decomposition of an adsorption complex formed between the developer and silver ions. This hypothesis was amplified by Sheppard and Meyer in the following year (95). Volmer showed that the oxygen oxidation of several developing agents is accelerated by colloidal silver, and likewise suggested a catalyzing action of silver in development (127, 128). However, at least in the case of hydroquinone, it appears that adsorption of oxygen to the silver is responsible for the effect Volmer observed, rather than any activation of the developing agent (*cf.* 3, 4). Rabinovitch suggested adsorption of the developing agent to silver as the basis for the catalysis in development. His data have been considered in a preceding section and shown to be inadequate. It is possible that adsorption of developing agent to silver plays a kinetic rôle in some cases, such as that of hydroxylamine, but experimental results with hydroquinone show that it cannot be considered the fundamental reason for development.

The real key to the fundamental mechanism of development probably lies in a suggestion made by Sheppard in 1929: "The importance of a suitable nucleus, e.g., silver or silver sulfide . . . becomes twofold. On the one hand, its interface with the silver halide provides the necessary ionic deformation for reactivity; on the other hand, it provides a break in the adsorption layer, a platform for displacement processes" (104). In some interface reactions, it is probable that the reaction product acts merely as a crystallization center, but in many reactions involving true catalysis, a reduction of the activation energy is strongly indicated. The boundaries at which these reactions occur are the "lines of especially unsaturated character at which synergetic effects are to be expected. That this is so is directly supported by other observations; the reaction product can often be replaced by other substances" (88). Thus, the thermal decomposition of silver oxide, in addition to being accelerated by silver and platinum which could act as crystallization nuclei, is accelerated by manganese which could not; the decomposition of mercuric oxide and barium peroxide is accelerated by a series of other oxides (117); the decomposition of silver permanganate is even accelerated by powdered pumice stone.

The observations of Wischin on the thermal decomposition of crystals of barium azide (151) include data on the energy of activation both of nuclear formation and of growth. The rate of growth of each nucleus follows the law:

$$\mathrm{d}r/\mathrm{d}t = B$$

where r is the radius and B is dependent only upon the temperature. The energy of activation of nuclear growth is approximately 23.5 kcal., compared with 74 kcal. for nuclear formation.

In the development process an external molecule, the developing agent, is brought into reaction at the interface. The basic mechanism of the silver catalysis in this reaction and in the silver-catalyzed reduction of silver ions from solution is essentially the same. It will be simpler to treat the latter first.

Evidence from several sources showing rather strong adsorption of silver ions to the silver has already been discussed. The reaction system may be treated (*cf.* 56) as consisting of three reactants—the silver ions, the developer, and the adsorption site on the surface of the catalyst; thus:

$$\mathrm{D} + \mathrm{Ag}^{\bullet} + \mathrm{S} \rightleftharpoons (\mathrm{D-Ag}^{\bullet}\mathrm{-S})^{\ddagger} \rightarrow \text{products} \quad (5)$$

The difference between the energy of activation required for this catalyzed reaction and that required for homogeneous reaction will equal the difference in potential energy of the activated states $(\mathrm{D-Ag}^{\bullet}\mathrm{-S})^{\ddagger}$ and $(\mathrm{D-Ag}^{\bullet})^{\ddagger}$, i.e., the *heat of adsorption* of the activated complex minus the heat of adsorption of the reactants. This is illustrated schematically in figure 7. Here, the solid curve refers to the homogeneous reaction, the broken curve to the heterogeneous reaction; E_{hom} is the activation energy of the homogeneous reaction; E_{a} is the apparent or measured energy of activation of the heterogeneous process, whereas E_{het} is the true activation energy; E_{a} differs from E_{het} by the heat of adsorption, λ, i.e., $E_{\mathrm{a}} = E_{\mathrm{het}} - \lambda$.

At the silver–silver halide interface, the silver ions are already present in an "adsorbed" condition. The significant difference is imposed by the presence of the silver halide lattice. The concentration of the activated complex will depend upon the availability of the silver ions for combination with the developer, and this, in turn, will depend upon the lattice forces. In addition, external materials strongly adsorbed to the halide, such as gelatin or the cyanine dyes, will greatly decrease the concentration of activated complex, as will adsorbed excess halide ion. This effect, due either to adsorption already involving the silver ions or to a steric shielding of them, is quite distinct from the electrical repulsion effect previously discussed and is operative with the uncharged developers as well as with the charged ones.

Differences are to be expected between the physical form of the silver obtained by reduction of the solid silver halides and that obtained by the silver-catalyzed reduction of silver ions from solution. In the latter case, the silver ions and reducing agents have free access to all parts of the silver surface, and the particles grow in a uniform manner. In the former case, however, free contact of the reducing agent is attained only at the triple interface between silver, silver halide, and solution. The silver formed in the reaction does not occupy as much space, if it forms a compact lattice, as did the original silver bromide. As reaction proceeds and bromide ions pass into solution, the silver-silver halide interface will progress in an erratic fashion, depending upon the presence of flaws in the crystal structure, accessibility of the interface to the developer, and localized breaks in the interface due to differences in space requirements. Reaction proceeding chiefly along surfaces and producing silver filaments within the approximate confines of the original grain, such as is revealed by electron micrographs, is to be expected on this basis. Filaments projecting beyond the original confines of the grain probably arise from other causes, although the silver filaments in the absence of rigid support (as is the case in the electron microscope technique) may possess considerable mobility.

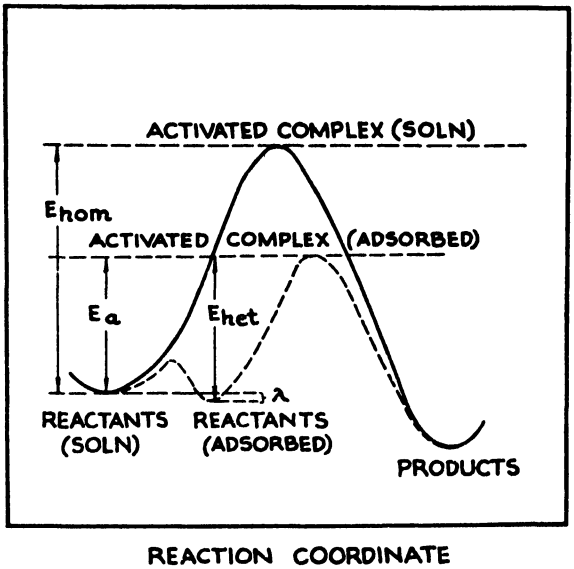


Fig. 7. Comparison of activation energies for homogeneous and heterogeneous reactions

An auxiliary factor which may markedly influence the final physical result in some cases arises from the finite solubility of the silver halides. This solubility is enhanced by the sodium sulfite present in most conventional developers and by additions such as potassium thiocyanate which are made to the "fine-grain" developers. These solvents will not increase the *equilibrium* silver-ion concentration attainable, but they will increase considerably the rate at which silver ions may pass into solution and, in addition, their action will expose silver nuclei existing within the original grains. Competition between the silver-catalyzed reduction of silver ions from solution and the interface reaction is to be expected in all cases. The former reaction may be negligible, as in the case of the pure hydroquinone developers previously discussed. It may also be large, as indicated by microscopic studies of the action of the fine-grain developers (62). The fundamental basis of development is not changed in any case. Only the practical result is altered.

Further complications may arise, owing to the localized solvent action of excess halide ion which is liberated around the development centers during reduction. Such action may be responsible for the streamers of silver observed under the microscope when perfect crystals of silver bromide are attacked by a rapidly acting developer, such as a metal–hydroquinone developer containing carbonate. A slowly acting developer, such as that used by Yano (153), does not produce the streamers. This effect offers one of the explanations for the filaments which, under the electron microscope, are observed to project out from the grain surface.

The amount of experimental material on the mechanism of fog formation, i.e., the unselective reduction of the silver halide, is very small. However, on the basis of the experimental material already given, the factors leading to fog formation can be foreseen. The reduction of silver ions in solution not catalyzed by silver will be independent of light exposure. In time, this will lead to the formation of nuclei which can then grow by silver-catalyzed reduction of silver ions. Fog silver produced in this way will have a smaller covering power than image silver and may even be of different color. A direct attack of the developing agent on the grain occurs, as evidenced by the reduction of pure silver chloride by hydroxylamine. Nuclei are thus formed which can catalyze the continued reduction of the grain (development fog). Catalytic nuclei may already exist in some grains prior to exposure as a result of reactions during the ripening process (emulsion fog). Finally, any additional reaction which can give rise to catalytic nuclei, such as the interaction of silver ions and sulfide, will lead to fog. Successful development depends upon adequate control of all of these factors, so that the rate of the fogging reactions is small compared with that of normal development.

The direct attack of the developing agent upon the unexposed silver halide forms the basis for a special type of developing action. The experiments with hydroxylamine and pure silver chloride showed clearly that the rate of initiation of reduction is markedly decreased by adsorption of protective substances, such as the cyanine dyes, gelatin, and chloride itself. Any abrasive action which tears such a layer from a portion of the surface, or splits the crystals and thereby exposes a fresh surface, causes an increase in the rate of initiation of reduction, provided conditions do not permit a rapid and complete reëstablishment of the productive layer. On this basis, the Poindexter effect and the production of a developable image by abrasion are easily understood. A silver nucleus is not required initially, and development arises from a cause which is qualitatively distinct from the more usual one.

REFERENCES

(1) Ardenne, M. von: Z. angew. Phot. **2,** 14 (1940).
(2) Arens, H.: Z. wiss. Phot. **30,** 127 (1931).
(3) Benton, A. F., and Bell, R. T.: J. Am. Chem. Soc. **56,** 501 (1934).
(4) Benton, A. F., and Drake, L. C.: J. Am. Chem. Soc. **56,** 255 (1934).
(5) Berg, W. F., and Mendelssohn, K.: Proc. Roy. Soc. (London) **A168,** 168 (1938).
(6) Berg, W. F.: Trans. Faraday Soc. **35,** 445 (1939).
(7) Berg, W. F., Marriage, A., and Stevens, G. W. W.: J. Optical Soc. Am. **31,** 385 (1940).
(8) Beukers, M. C. F.: "Fotografische Ontwikkelaars", Dissertation, Delft, 1934.
(9) Blau, M., and Wambacher, H.: Z. wiss. Phot. **31,** 243 (1933).
(10) Branch, G. E. K., and Joslyn, M. A.: J. Am. Chem. Soc. **57,** 2388 (1935).
(11) Broili, H., and Kiessig, H.: Z. Physik **87,** 425 (1934).
(12) Carroll, B. H., and Hubbard, D.: Bur. Standards J. Research **1,** 565 (1928).
(13) Carroll, B. H., and Hubbard, D.: Bur. Standards J. Research **12,** 329 (1934).
(14) Clark, W.: Brit. J. Phot. **69,** 462 (1922).
(15) Clark, W.: Phot. J. **63,** 230 (1923).
(16) Clark, W.: Brit. J. Phot. **70,** 763 (1923).
(17) Clark, W.: Brit. J. Phot. **70,** 717 (1923).
(18) Clark, W.: Trans. Faraday Soc. **19,** 309 (1923).
(19) Dombrowsky, C.: Dissertation, Leipzig, 1908.
(20) Eggert, J., and Noddack, W.: Z. Physik **20,** 299 (1923).
(21) Eggert, J., and Noddack, W.: Z. Physik **31,** 922 (1925).
(22) Euler, H. von: Z. Elektrochem. **28,** 446 (1922).
(23) Fischbeck, K., and Schnaidt, K.: Z. Elektrochem. **38,** 299 (1932).
(24) Gall, H., and Roth, H.: Z. anorg. Chem. **214,** 201 (1933).
(25) Gee, G.: J. Chem. Phys. **5,** 801 (1937).
(26) Glocker, R., and Traub, W.: Physik. Z. **22,** 345 (1921).
(27) Günther, P., and Tittel, H.: Z. Elektrochem. **39,** 646 (1933).
(28) Gurney, R. W., and Mott, N. F.: Proc. Roy. Soc. (London) **A164,** 151 (1938).
(29) Haber, F., and Willstätter, R.: Ber. **64,** 2844 (1931).
(30) Hall, C. E., and Schoen, A. L.: J. Optical Soc. Am. **31,** 281 (1941).
(31) Higson, G. J.: Phot. J. **60,** 161 (1920).
(32) Hirsh, F. R., Jr.: J. Optical Soc. Am. **25,** 229 (1935).
(33) Hirsh, F. R., Jr.: J. Optical Soc. Am. **28,** 463 (1938).
(34) Hodgson, M. B.: J. Franklin Inst. **184,** 705 (1917).
(35) Huggins, M. L.: Private communication.
(36) Hurter, F., and Driffield, V. C.: J. Soc. Chem. Ind. **9,** 455 (1890).
(37) Jablczynski, K., and Kobryner, S.: Roczniki Chem. **9,** 715 (1929).
(38) James, T. H., and Weissberger, A.: J. Am. Chem. Soc. **60,** 98 (1938).
(39) James, T. H., Snell, J. M., and Weissberger, A.: J. Am. Chem. Soc. **60,** 2084 (1938).
(40) James, T. H., and Weissberger, A.: J. Am. Chem. Soc. **61,** 442 (1939).
(41) James, T. H.: J. Am. Chem. Soc. **61,** 648 (1939).
(42) James, T. H.: J. Am. Chem. Soc. **61,** 2379 (1939).
(43) James, T. H.: J. Phys. Chem. **43,** 701 (1939).
(44) James, T. H.: J. Phys. Chem. **44,** 42 (1940).
(45) James, T. H.: J. Am. Chem. Soc. **62,** 536 (1940).
(46) James, T. H.: J. Am. Chem. Soc. **62,** 1649 (1940).
(47) James, T. H.: J. Am. Chem. Soc. **62,** 1654 (1940).
(48) James, T. H.: J. Am. Chem. Soc. **62,** 3411 (1940).
(49) James, T. H.: J. Phys. Chem. **45,** 223 (1941).
(50) James, T. H.: J. Am. Chem. Soc. **63,** 1601 (1941).
(51) Jost, W.: Trans. Faraday Soc. **34,** 860 (1938).
(52) Kempf, A.: Z. wiss. Phot. **36,** 235 (1937).

(53) Kornfeld, G., and Weissberger, A.: J. Am. Chem. Soc. **61,** 360 (1939).
(54) Kornfeld, G.: J. Optical Soc. Am. **31,** 598 (1941).
(55) Kreveld, A. van, and Jürriens, H. J.: Physica (IV) **4,** 297 (1937).
(56) Laider, K. J., Glasstone, S., and Eyring, H.: J. Chem. Phys. **8,** 667 (1940).
(57) deLanghe, J. E.: Z. wiss. Phot. **35,** 201 (1936).
(58) Langmuir, I.: J. Am. Chem. Soc. **38,** 2263 (1916).
(59) Livingston, R., and Lingane, J. J.: J. Chem. Education **15,** 320 (1938).
(60) Lottermoser, A., and Steudel, R.: Kolloid-Z. **82,** 319 (1938).
(61) Lottermoser, A., and Steudel, R.: Kolloid-Z. **83,** 37 (1938).
(62) Loveland, R. P.: Private communication.
(63) Lüppo-Cramer, H.: Kolloid-Z. **15,** 164 (1914).
(64) Lüppo-Cramer, H.: *Kolloidchemie und Photographie,* 2nd edition. T. Steinkopff, Dresden (1921).
(65) Luther, R., and Leubner, A.: Brit. J. Phot. **59,** 632 *et seq.* (1912).
(66) McEwen, W. K.: J. Am. Chem. Soc. **58,** 1124 (1936).
(67) Meidinger, W.: Z. physik. Chem. **114,** 89 (1924).
(68) Meidinger, W.: Physik. Z. **36,** 312 (1935).
(69) Meidinger, W.: Phot. Ind. **34,** 1305 (1936).
(70) Meidinger, W.: Physik. Z. **38,** 564, 737, 905 (1937).
(71) Michaelis, L.: Chem. Rev. **16,** 243 (1935).
(72) Michaelis, L., and Schubert, M. P.: Chem. Rev. **22,** 437 (1938).
(73) Michaelis, L., Schubert, M. P., Reber, R. K., Kuck, J. A., and Granick, S.: J. Am. Chem. Soc. **60,** 1678 (1938).
(74) Mott, N. F.: Trans. Faraday Soc. **34,** 822 (1938).
(75) Mott, N. F.: Reports on Progress in Physics **6,** 186 (1939).
(76) Mott, N. F., and Gurney, R. W.: *Electronic Processes in Ionic Crystals.* The Clarendon Press, Oxford (1940).
(77) Mülbach, E.: Z. wiss. Phot. **36,** 269 (1937).
(78) Nutting, P. G.: Phil. Mag. **26,** 421 (1913).
(79) Ollendorff, G., and Rhodius, R.: Z. wiss. Phot. **35,** 81 (1936).
(80) Piper, C. W.: Brit. J. Phot. **55,** 196 (1908).
(81) Proskurnin, M., and Frumkin, A.: Z. physik. Chem. **A155,** 29 (1931).
(82) Rabinovitch, A. J., and Peissachovitch, S.: Z. wiss. Phot. **33,** 94 (1934).
(83) Rabinovitch, A. J.: (a) Trans. Faraday Soc. **34,** 920 (1938); (b) Photo Kino Chem. Ind. (U.S.S.R.) **3** (1938).
(84) Reinders, W., and Hamburger, L.: Z. wiss. Phot. **31,** 32, 265 (1932).
(85) Reinders, W., and deVries, R. W. P.: Rec. trav. chim. **56,** 985 (1937).
(86) Scheffer, W.: Brit. J. Phot. **54,** 116, 271 (1907).
(87) Scheffers, H.: Z. Physik **20,** 120 (1923).
(88) Schwab, G. M., Taylor, H. S., and Spence, R.: *Catalysis.* D. Van Nostrand Company, New York (1937).
(89) Schwarz, G., and Urbach, F.: Z. wiss. Phot. **31,** 79 (1932).
(90) Schwarz, G., and Urbach, F.: Phot. Korr. **68,** 61 (1932).
(91) Schwarz, G.: Phot. Korr. **69,** 27 (1933).
(92) Sheppard, S. E., and Mees, C. E. K.: Proc. Roy. Soc. (London) **A76,** 217 (1905).
(93) Sheppard, S. E., and Mees, C. E. K.: *Investigations on the Theory of the Photographic Process.* Longmans, Green and Company, London (1907).
(94) Sheppard, S. E.: Phot. J. **59,** 135 (1919).
(95) Sheppard, S. E., and Meyer, G.: J. Am. Chem. Soc. **42,** 689 (1920).
(96) Sheppard, S. E., Elliott, F. A., and Sweet, S. S.: Trans. Faraday Soc. **19,** 261 (1923).
(97) Sheppard, S. E., and Wightman, E. P.: J. Franklin Inst. **195,** 337 (1923).
(98) Sheppard, S. E., Wightman, E. P., and Trivelli, A. P. H.: J. Franklin Inst. **196,** 653, 779 (1923).

(99) Sheppard, S. E., Wightman, E. P., and Trivelli, A. P. H.: J. Franklin Inst. **198,** 507 (1924).

(100) Sheppard, S. E., Wightman, E. P., and Trivelli, A. P. H.: J. Franklin Inst. **198,** 629 (1924).

(101) Sheppard, S. E., Trivelli, A. P. H., and Loveland, R. P.: J. Franklin Inst. **200,** 51 (1925).

(102) Sheppard, S. E.: Phot. J. **65,** 380 (1925).

(103) Sheppard, S. E., and Ballard, A.: J. Franklin Inst. **205,** 659 (1928).

(103a) Sheppard, S. E., and Trivelli, A. P. H.: Phot. Korr. **64,** 145, 173, 242, 273 (1928).

(104) Sheppard, S. E.: Phot. J. **69,** 330 (1929).

(105) Sheppard, S. E., Vanselow, W., and Hall, V. C.: J. Phys. Chem. **33,** 1403 (1929).

(106) Sheppard, S. E.: Phot. J. **71,** 313 (1931).

(107) Sheppard, S. E., Lambert, R. H., and Keenan, R. L.: J. Phys. Chem. **36,** 174 (1932).

(108) Sheppard, S. E., Vanselow, W., and Happ, G. P.: J. Phys. Chem. **44,** 411 (1940).

(109) Sheppard, S. E., Ballard, A., and Perry, E. S.: J. Am. Chem. Soc. **63,** 2357 (1941).

(110) Shishkina, N. N.: J. Phys. Chem. (U. S. S. R.) **10,** 848 (1937).

(111) Silberstein, L., and Trivelli, A. P. H.: J. Optical Soc. Am. **28,** 441 (1938).

(112) Silberstein, L.: J. Optical Soc. Am. **31,** 343 (1941).

(113) Staude, H.: Fot. Rundschau **72,** 390 (1935).

(114) Staude, H.: Z. wiss. Phot. **38,** 65 (1939).

(115) Staude, H.: Z. wiss. Phot. **38,** 137 (1939).

(116) Svedberg, T.: Z. wiss. Phot. **20,** 36 (1920).

(116a) Svedberg, T., and Andersson, H.: Phot. J. **61,** 325 (1921).

(117) Taylor, H. S.: J. Phys. Chem. **28,** 933 (1924).

(118) Toy, F. C.: Phot. J. **61,** 417 (1921).

(119) Toy, F. C.: Nature **120,** 441 (1927).

(120) Toy, F. C., and Harrison, G. B.: Proc. Roy. Soc. (London) **A127,** 613 (1930).

(121) Trivelli, A. P. H., and Sheppard, S. E.: *The Silver Bromide Grain of Photographic Emulsions*, Monograph No. 1 on the Theory of Photography from the Research Laboratories of the Eastman Kodak Co., Rochester, New York (1921).

(122) Trivelli, A. P. H., Righter, F. L., and Sheppard, S. E.: Phot. J. **62,** 407 (1922).

(123) Tuttle, C., and Trivelli, A. P. H.: Phot. J. **68,** 465 (1928).

(124) Urbach, F.: Proc. IX Intern. Congr. Phot., Paris, p. 432 (1935).

(125) Vanselow, W., and Sheppard, S. E.: J. Phys. Chem. **33,** 331 (1929).

(126) Veselovsky, V. I.: Acta Physicochim. U. R. S. S. **11,** 815 (1939).

(127) Volmer, M.: Z. wiss. Phot. **20,** 189 (1921).

(128) Volmer, M.: Phot. Korr. **58,** 226 (1921).

(129) Wagner, C.: Trans. Faraday Soc. **34,** 851 (1938).

(130) Webb, J. H.: J. Optical Soc. Am. **23,** 157 (1933).

(131) Webb, J. H.: J. Optical Soc. Am. **25,** 4 (1935).

(132) Webb, J. H.: J. Optical Soc. Am. **26,** 347 (1936).

(133) Webb, J. H.: J. Optical Soc. Am. **26,** 367 (1936).

(134) Webb, J. H., and Evans, C. H.: J. Optical Soc. Am. **28,** 249 (1938).

(135) Webb, J. H., and Evans, C. H.: J. Optical Soc. Am. **28,** 431 (1938).

(136) Webb, J. H.: J. Optical Soc. Am. **29,** 309 (1939).

(137) Webb, J. H.: J. Optical Soc. Am. **29,** 314 (1939).

(138) Webb, J. H.: J. Applied Phys. **11,** 18 (1940).

(139) Webb, J. H., and Evans, C. H.: J. Optical Soc. Am. **30,** 445 (1940).

(140) Webb, J. H.: J. Optical Soc. Am. **31,** 348 (1941).

(141) Webb, J. H., and Evans, C. H.: J. Optical Soc. Am. **31,** 355 (1941).

(142) Webb, J. H.: J. Optical Soc. Am. **31,** 559 (1941).

(143) Wightman, E. P., and Sheppard, S. E.: J. Phys. Chem. **25,** 181 (1921).

(144) Wightman, E. P., and Sheppard, S. E.: J. Phys. Chem. **25,** 561 (1921).

(145) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: J. Franklin Inst. **194,** 485 (1922).
(146) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: J. Phys. Chem. **27,** 1 (1923).
(147) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: J. Phys. Chem. **27,** 141 (1923).
(148) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: J. Phys. Chem. **27,** 466 (1923).
(149) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: Trans. Faraday. Soc. **19,** 270 (1923).
(150) Wightman, E. P., Trivelli, A. P. H., and Sheppard, S. E.: J. Phys. Chem. **28,** 529 (1924).
(151) Wischin, A.: Proc. Roy. Soc. (London) **A172,** 314 (1939).
(152) Wulff, P., and Seidl, K.: Z. wiss. Phot. **28,** 239 (1930).
(153) Yano, T.: J. Soc. Chem. Ind. Japan (Suppl. Bind.) **43,** 44B (1940).

In addition to the specific references, the following books may be consulted:
Mees, C. E. K.: *The Theory of the Photographic Process.* The Macmillan Company, New York (1942).
Meidinger, W.: *Handbuch der wissenschaftlichen und angewandten Photographie,* Vol. V. J. Springer, Berlin (1932).

THE CONSTITUENTS OF DERRIS AND OTHER ROTENONE-BEARING PLANTS

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I. INTRODUCTION

The wide interest in *Derris*, *Lonchocarpus*, *Tephrosia*, *Mundulea*, and other rotenone-bearing plants is attested by the many articles (64) relating to them that have appeared in the literature during the past fifteen years. The roots of these plants, all closely related botanically and belonging to the family *Fabaceae*, are used in large quantities for the preparation of dusts and sprays for combating many injurious insects.

More than six and a half million pounds of *Derris* and *Lonchocarpus*,[1] the most important genera commercially, were imported into the United States in 1940. The former is obtained from British Malaya and the Dutch East Indies and the latter from South America.

The rotenone-bearing plants have been shown to be toxic to many widely different species of insects (65). McIndoo, Sievers, and Abbott (57), in an extensive study of derris as an insecticide, have demonstrated that it acts both as a contact insecticide and as a stomach poison. Tischler's studies (77) on the physiological mode of toxicity of derris to insects have been summarized by Holman (40) as follows:

> ". . . (1) that extracts enter the insect's body by (a) the alimentary canal, through (b) spiracles and tracheal system, and (c) through the integument, and that there is for certain insects a definite stomach-poisoning effect; (2) that dusts penetrate by (a) and (c), but probably not by (b); (3) that body exudates and body fluids may extract the poison, that there are thin surface areas through which osmosis can take place, and that derris dust settling on these (areas containing body exudates) finds continuous access to the body until death takes place; (4) that there are no specific effects on motor nerves and attached muscles, and that circulation effects are secondary in character; (5) that derris acts primarily by the inhibition of oxygen utilization, and that this effect is general rather than specific to any organ or system."

Derris and allied materials in large doses exercise some toxic action upon man and higher animals, but they are far less toxic than the arsenicals which are used for insecticidal purposes. Haag (32) considered that there was little danger of acute poisoning to healthy subjects following the ingestion of derris on foods, but workers in derris dusts should be protected by respirators.

The insecticidal action of the roots was attributed at first to a single constituent, rotenone (52, 62). This compound separates in optically active, crystalline form when extracts of the roots, made with suitable organic solvents, are concentrated. The extracts contain a number of related compounds other than rotenone, which are obtained as an uncrystallizable residue after complete evaporation of the solvent. The percentage of total extractives, as well as the proportion of rotenone to total extractives, varies widely and depends upon several factors, such as the species, the method of cultivation, and the solvent used in the extraction. In the case of derris, which has been most extensively studied, the residue, when freed from rotenone as completely as possible, is known as derris resin.[2] The resins, like rotenone, are usually optically active and most of them are levorotatory.

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[1] Commonly called cube, barbasco, haiari, timbo, and nekoe. These names, however, are not only applied to *Lonchocarpus* but are used for other entirely unrelated fish-poison plants, many of which are of no insecticidal value.

In 1930 Clark (14) found that derris resin, on treatment with alkali, yielded several crystalline optically inactive substances, which he named deguelin, tephrosin, and toxicarol. Tests on all three compounds against aphids by Davidson (23), against silkworms by Shepard and Campbell (68), and against mosquito larvae by Campbell and Sullivan (unpublished data) indicated that deguelin was much less toxic than rotenone, while tephrosin and toxicarol were practically non-toxic. From these results the erroneous conclusion was drawn that the extractives other than rotenone had little or no insecticidal value, and the toxicity of the derris resin was ascribed to its occluded rotenone. Various comparative tests of derris extractives and rotenone, however, have indicated that toxic substances other than rotenone must be present in the extractives. For example, Jones *et al.* (44) found that powdered derris extractives that contained about 25 per cent of rotenone were as toxic to mosquito larvae as was pure rotenone, and Campbell *et al.* (13) found that a kerosene extract of derris from which no rotenone could be isolated was effective against houseflies. These results, as well as those of Tattersfield and Martin (56, 73), have led to further intensive investigations of derris resin from a number of species and varieties of *Derris* root. Studies have also been made on other rotenone-bearing legumes, of which the most important are species of *Tephrosia* and *Lonchocarpus.*

The isolation of rotenone by Clark (21) from the roots of *Tephrosia virginiana* L., the most abundant species of *Tephrosia* indigenous to the United States, stimulated interest in this plant as a source of insecticides. Analytical studies (43) of samples of this plant, commonly known as devil's shoestring, have shown considerable variation in the amount of insecticidal constituents, but recent selective breeding experiments (55, 69) make it now possible to produce a root of uniformly high quality. Limited quantities of these new varieties have been available for studies on their chemical constituents (29).

II. CHEMISTRY OF ROTENONE AND RELATED COMPOUNDS

A. Rotenone

Through the efforts of three groups of chemists the structural formula for rotenone is now unreservedly accepted to be I, and for an understanding of the fundamentals of its chemistry the reader is referred to reviews by LaForge, Haller, and Smith (50), Butenandt and McCartney (8), and King (48).

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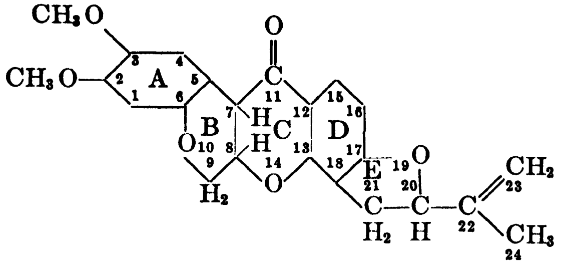
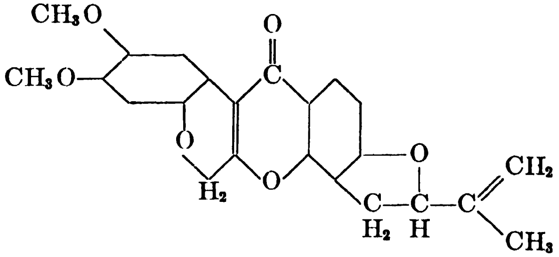
[2] The term "deguelin concentrate" has also been used, but we regard this term as less appropriate.

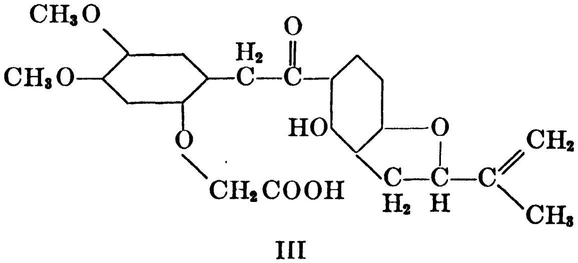


I
Rotenone

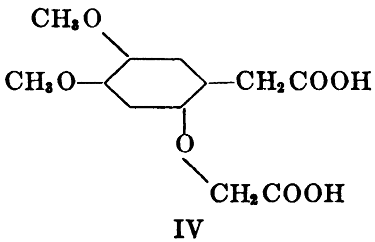
One of the reactions of rotenone, common to most of the compounds isolated from the uncrystallizable fraction of the extractives, is the loss of two hydrogen atoms on gentle oxidation, with the formation of dehydrorotenone (II) (6). On treatment with alcoholic potash this compound adds two molecules of water to form derrisic acid (III) (53), which when oxidized with hydrogen peroxide yields derric acid (IV). With potassium permanganate derric acid yields a



II
Dehydrorotenone

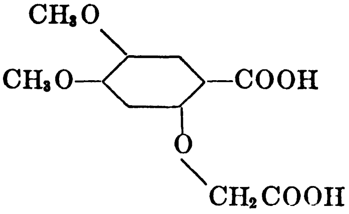


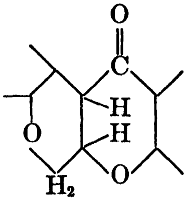
III
Derrisic acid



IV
Derric acid

lower homolog, risic acid (V) (54). This sequence of reaction is shown by all the compounds having a chromane–chromanone ring system (VI).

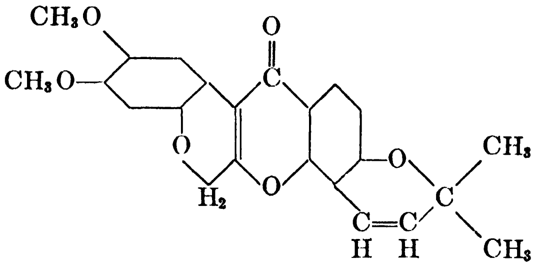

V
Risic acid

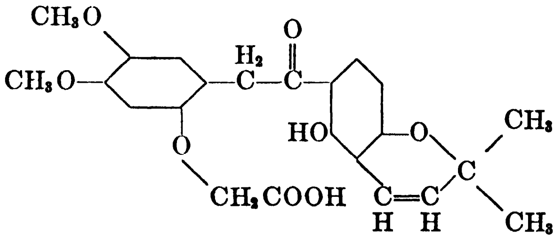

VI
Chromane–chromanone system

B. *Deguelin*

Deguelin was first described by Clark (16), who obtained it in optically inactive crystalline form on treatment of an alcoholic solution of derris resin with alkali.[3] It has also been obtained from cube, *Tephrosia*, etc.

Deguelin has the molecular formula $C_{23}H_{22}O_6$, and is thus isomeric with rotenone. Like the latter it possesses two methoxyl groups and with mild oxidizing agents yields the dehydro compound, dehydrodeguelin (VII) (16), which on treatment with alcoholic alkali adds two molecules of water with the formation of deguelic acid (VIII). Oxidation of this compound in alkaline solution with


VII
Dehydrodeguelin


VIII
Deguelic acid

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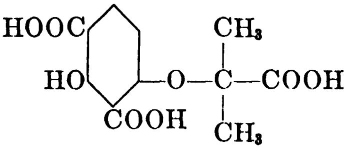
[3] The isodeguelin obtained by Merz (58) from the seeds of *Cracca vogelii* has been shown by Boam, Cahn, and Stuart (3) to be identical with deguelin.

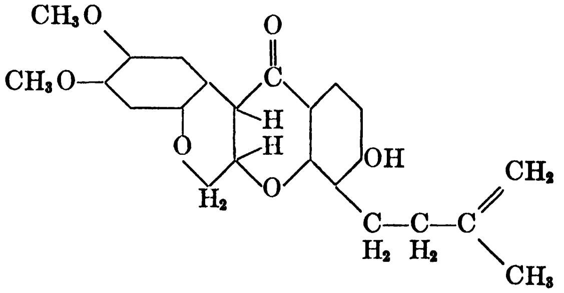
hydrogen peroxide yields derric acid (IV). It thus follows that in deguelin, as in rotenone, derric acid is derived from one half of the molecule, and hence the structural differences between deguelin and rotenone are to be found in the second half of the molecule or in the linkages between the two parts.

Oxidation of dehydrodeguelin with potassium permanganate yields, besides 2-hydroxy-4,5-dimethoxybenzoic acid and risic acid, nicouic acid (18). Nicouic acid is a tricarboxylic acid having the structure represented by formula IX.

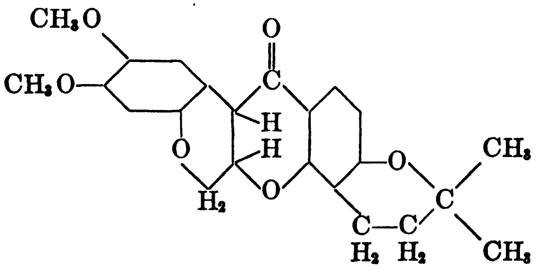


IX
Nicouic acid

The absence of methoxyl groups shows it to be derived from the undetermined half of the deguelin molecule. The isolation of this acid, together with the fact that rotenonic acid (isodihydrorotenone) (X), which is obtained on catalytic hydrogenation of rotenone, is isomerized with sulfuric acid to β-dihydrorotenone (XI) (33), the optically inactive form of which is identical with dihydrodeguelin,

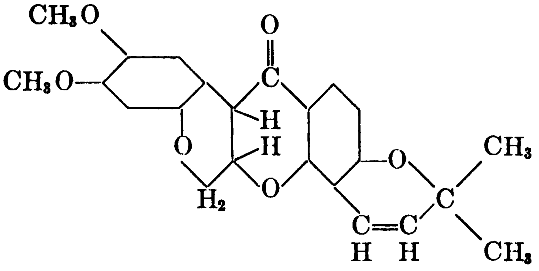


X
Rotenonic acid



XI
β-Dihydrorotenone (dihydrodeguelin)

showed deguelin to possess the structural formula XII. This structure differs

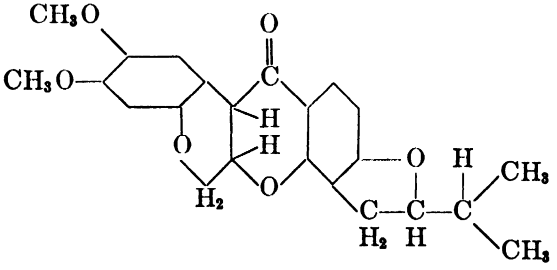


XII

Deguelin

from that of rotenone in that ring E is a substituted chromene, whereas in rotenone it is a substituted dihydrofuran. Also rotenone possesses three asymmetric carbon atoms, numbers 7, 8, and 20, while deguelin has but two, numbers 7 and 8. Carbon atoms 7 and 8 may be regarded as one asymmetric center and carbon atom 20 as another. The effect of alkali on these centers has recently been studied by Cahn *et al.* (11). Like rotenone (41, 45) deguelin crystallizes from certain organic liquids with a definite molecular ratio of solvent of crystallization (27). Gravimetric methods for the determination of deguelin have been proposed by Takei *et al.* (71) and by Goodhue and Haller (28).

The relatively low insecticidal action of deguelin (23, 68) stimulated studies on its precursor. Haller and LaForge (34) found that, on hydrogenation of derris resin in the absence of alkali, optically active dihydrodeguelin (β-dihydrorotenone) was obtained. This compound possesses an insecticidal value of the same order as dihydrorotenone (XIII) and rotenone (25, 70). The type of hydrogenation procedure used made it appear probable that at least part of the deguelin was present in the resin in an optically active form. The conclusions of Haller and LaForge have since received support by the work of Tattersfield and Martin (74), Cahn and his collaborators (12), and Harper (36).

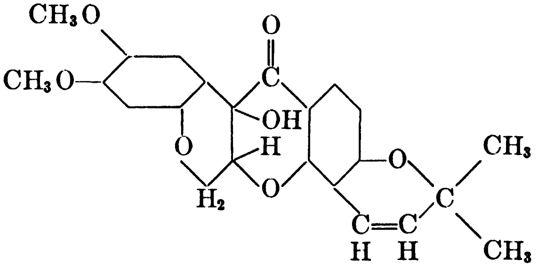


XIII

Dihydrorotenone

C. Tephrosin

Hanriot (35) in 1907 isolated from the leaves of *Tephrosia vogelii* a substance which he named tephrosin. Similar results were obtained in 1915 by an anonymous worker (1), and in 1926 Tattersfield *et al.* (72) obtained two apparently different substances[4] having higher melting points than Hanriot's product. Clark (17) obtained a crystalline material corresponding to that described by Hanriot from the same source and showed it to be a mixture of two compounds. One was deguelin and the other a colorless crystalline substance that Clark designated tephrosin. Clark also isolated this compound from the mixture of crystalline substances obtained on treatment of derris resin in alcohol with alkali. He showed that it had the molecular formula $C_{23}H_{22}O_7$, was optically inactive, and possessed two methoxyl groups. On treatment with acetic anhydride or acetic and sulfuric acids, tephrosin loses a molecule of water and dehydrodeguelin (VII) is formed. It is thus clear that tephrosin (XIV) is a hydroxydeguelin. Since it has been shown that dehydrodeguelin results from deguelin by the loss of hydrogen atoms from carbon atoms 7 and 8, it follows that tephrosin possesses a hydroxyl group in place of a hydrogen atom on one of these two carbon atoms. No information is available, however, as to the exact configuration of tephrosin.



XIV
Tephrosin

An isomer of tephrosin, isotephrosin, has been isolated from a Peruvian cube root (22). With dehydrating agents it also yields dehydrodeguelin. Isotephrosin therefore is also a hydroxydeguelin and has the same relation to deguelin as has tephrosin. If the hydroxyl group of the latter is on carbon atom 7, then in isotephrosin it would be on carbon atom 8, or *vice versa.*

A third isomer of tephrosin, designated hydroxydeguelin-C (49), has also been described. It was prepared from deguelin by oxidation with hydrogen peroxide in alcoholic alkaline solution. It differs from tephrosin especially in its behavior toward dehydrating agents, for it was recovered unchanged after several hours' boiling with 8 per cent alcoholic sulfuric acid.

By the method of oxidation described above, isorotenone (XV), which is obtained on treatment of rotenone with mineral acid, yields two hydroxy compounds, designated isorotenone-C and isorotenone-D, both of which are stable to dehydrating agents.

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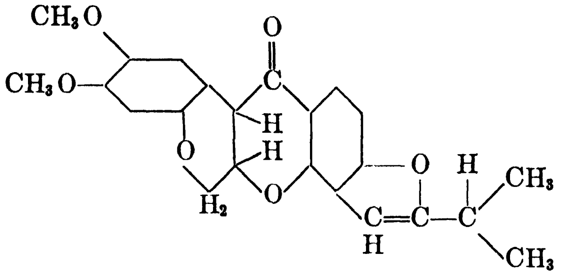
[4] Several years ago it was shown that one of the compounds was probably slightly impure tephrosin and the other a pure sample of tephrosin.



XV
Isorotenone

The two normal hydroxyisorotenones corresponding to tephrosin and isotephrosin have also been prepared. Both are readily dehydrated to the same dehydro compound (dehydroisorotenone). LaForge and Haller (49) have suggested that these isomers may be accounted for on the basis of spatial configuration, but this explanation has not met with ready acceptance.

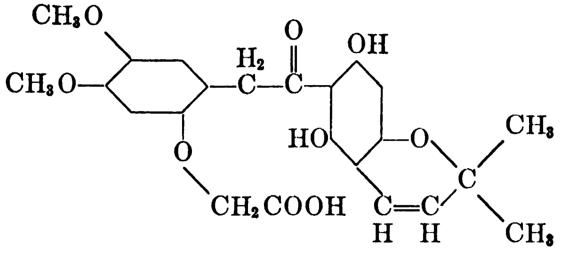
In 1933 Takei *et al.* (71) produced evidence that tephrosin does not occur as such in derris but is formed by oxidation of deguelin. These findings were supplemented by those of LaForge and Haller (49) that deguelin in alkaline solution is readily oxidized by air to a mixture of tephrosins, and those of Merz and Schmidt (59) that when a chloroform solution of deguelin is shaken with aqueous alkali it is partly oxidized to tephrosin. Cahn, Phipers, and Boam (12) also produced evidence that tephrosin is an oxidation product, and now all workers in this field seem agreed that tephrosin does not occur naturally in the resin. The allotephrosin and isoallotephrosin of Merz and Schmidt (59) have been shown to be identical with tephrosin (3).

D. Toxicarol

Clark (15) was the first to describe toxicarol. He obtained it in optically inactive form by alkali treatment of the extractives of the roots of *Tephrosia toxicaria* and from derris resin. Subsequently Cahn and Boam (9) showed that extracts of a certain species of derris giving no rotenone (63) by the usual carbon tetrachloride method nearly always yielded pure toxicarol on treatment with alkali. Because of its origin they have chosen to designate this kind of derris as being of the "Sumatra type." Such root has been referred to by Buckley (5) and by Harper (38) as "Kinta type" derris, because their specimens came from the Kinta district of Malaya.

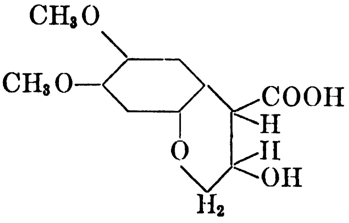
Toxicarol has the molecular formula $C_{23}H_{22}O_7$ (7, 15), being isomeric with tephrosin but, unlike it, possesses a phenolic hydroxyl group. Like rotenone it yields a dehydro compound (19) that adds two molecules of water with the formation of toxicarolic acid (XVI), and this in turn can be degraded to derric acid.

Thus it is shown that toxicarol possesses a chromane–chromanone nucleus of the rotenone type.

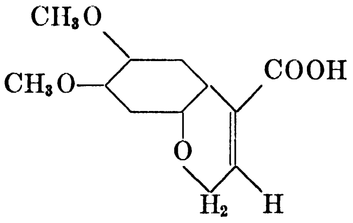


XVI
Toxicarolic acid

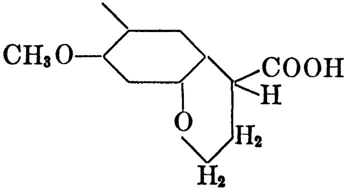
On treatment with alkali toxicarol loses C_5H_6, yielding apotoxicarol (20); this reaction is not shown by rotenone or deguelin. Apotoxicarol on oxidation in alkaline solution yields two monocarboxylic acids, hydroxynetoric acid (XVII) and dehydronetoric acid (XVIII). The former may be dehydrated to the latter, which on hydrogenation is reduced to netoric acid (XIX). Apotoxicarol undergoes the reactions characteristic of the chromane–chromanone residue and has been assigned formula XX. This structure has received confirmation by the synthesis of apotoxicarolic acid, which is formed on hydrolysis of dehydroapotoxicarol. Toxicarol and deguelin yield acetone on treatment with alkali under the proper conditions. This reaction indicates the grouping $(CH_3)_2C$ and supports the theory that toxicarol also contains the 2,2-dimethyl-Δ^3-



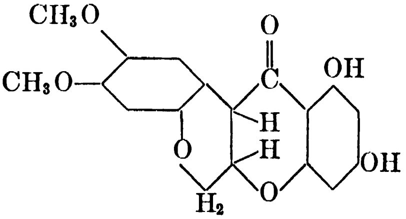
XVII
Hydroxynetoric acid



XVIII
Dehydronetoric acid

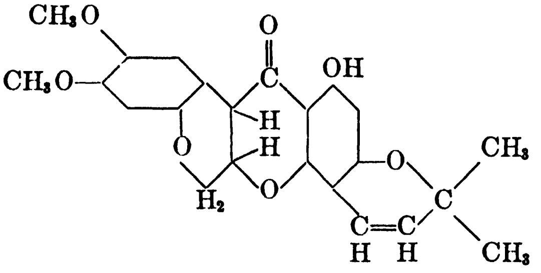


XIX
Netoric acid



XX
Apotoxicarol

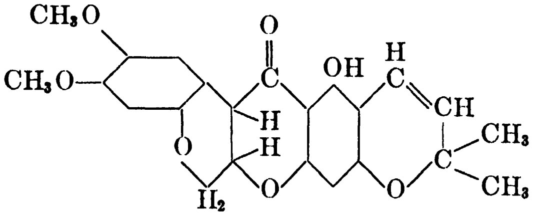
chromene residue characteristic of deguelin. On the basis of this evidence and by analogy, toxicarol has been tentatively assigned the formula XXI (26).



XXI
α-Toxicarol

Substantiation of the nature of the chromene system just postulated was furnished by Bridge *et al.* (4). Their evidence has recently been summarized by LaForge and Markwood (51).

When optically inactive toxicarol in acetone solution is heated with potassium carbonate, it is partly changed to an isomer, β-toxicarol (XXII) (11), the linear



XXII
β-Toxicarol

form of formula XXI. Its greater solubility in ether permits a separation of β-toxicarol from ordinary toxicarol (XXI), designated α-toxicarol. The reaction is reversible, but when alcoholic alkali is used the much less soluble α-form crystallizes out, so that in alcohol the change from the β-form to the α-form is almost complete. Usually, however, the α-form contains a small amount of the β-form, and this largely accounts for the various melting points reported for ordinary toxicarol: 213°C. by Butenandt and Hilgetag (7), 219°C. (corr.) by Clark (15), and 232–233°C. by Cahn, Phipers, and Boam (11), who have devised a method for purifying the α-form. Jones and Wood (46) have recently found that in capillary melting-point tubes made of soft-glass tubing the melting point of toxicarol is appreciably lower than in hard glass or Pyrex capillary tubes. The phenomenon has been shown to be due to the greater alkalinity of the soft-glass tubing.

Toxicarol that is obtained by the action of alkali on derris extractives is optically inactive. That it does not occur in this form in the root was first demonstrated by Tattersfield and Martin (75, 76). They succeeded in isolating from a Sumatra-type derris resin an optically active precursor, which was shown by Cahn *et al.* (11) to be *l*-α-toxicarol. The latter workers doubted the purity of Tattersfield's preparation, but Harper (38) has concluded that the optical data of Cahn *et al.* are untrustworthy and that their criticism of the work of Tattersfield and Martin is unjustified. The properties of the protoxicarol of Rowaan and Van Duuren (67), levorotatory in benzene and dextrorotatory in acetone, indicate it to be *l*-α-toxicarol.

Tattersfield and Martin (76) have shown the toxicarol precursor to be about one-fifteenth as toxic as rotenone to *Aphis rumicis* L. The Sumatra-type derris resin from which it was isolated was about one-sixth as toxic as rotenone or about half as toxic as samples of *Derris elliptica* resin.

E. Sumatrol

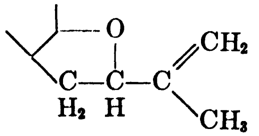
Sumatrol was first isolated by Cahn and Boam (10) from a Sumatra-type derris resin,[5] from which it was subsequently also obtained by other workers. It has not yet been found in *Derris elliptica.* While, according to Tattersfield and Martin (76), sumatrol is about as toxic as toxicarol to *Aphis rumicis*, it is present in the resin only in small amount and therefore is not regarded as an important contribution to the insecticidal effect of the resin.

Sumatrol is optically active and has the molecular formula $C_{23}H_{22}O_7$ (10), being isomeric with tephrosin and toxicarol. It differs from tephrosin in being phenolic and from toxicarol in being colorless.

Besides having a phenolic hydroxyl group, sumatrol, like all other members of the rotenone series, possesses two methoxyl groups, an active carbonyl group, and three chemically inactive oxygen atoms (66). Further, it yields a dehydro compound which furnishes an acid of the derrisic type by standard procedures. It therefore appears practically certain that sumatrol possesses the chromane–chromanone ring system which makes the chromene–chromone system capable of forming the derrisic acid type of compound.

The similarity of the colors given with ferric chloride and the insolubility in aqueous alkali suggested that sumatrol, like toxicarol, has a hydroxyl group in the 5-position of ring D.

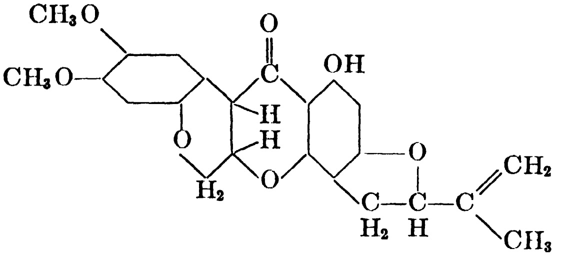
On hydrogenation sumatrol, like rotenone, yields a dihydro compound and a tetrahydro derivative. Both compounds are optically active and both form dehydro derivatives, but only the dihydrodehydro compound rotates the plane of polarized light. These facts are strong evidence that sumatrol, like rotenone,



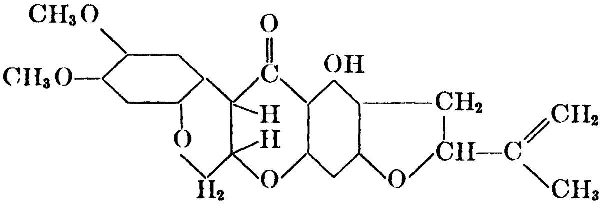
XXIII
Isopropenyldihydrofuran system

[5] The root was probably *Derris malaccensis* var. *sarawakensis.*

contains the system XXIII. Consequently it would appear that sumatrol is a hydroxyrotenone and may be represented by formula XXIV. The less likely linear formula XXV has also been considered (66).



XXIV
Sumatrol



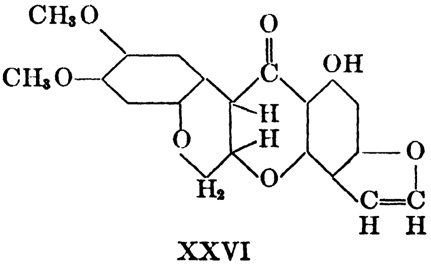
XXV
Sumatrol (linear formula)

The synthesis of tetrahydrosumatrolic acid and of dehydrotetrahydrosumatrol (47) substantiates the presence of the chromane–chromanone system in sumatorl and also makes certain the presence of the hydroxyl group in the 5-position of ring D, but it does not permit a final choice between formulas XXIV and XXV.

F. Malaccol

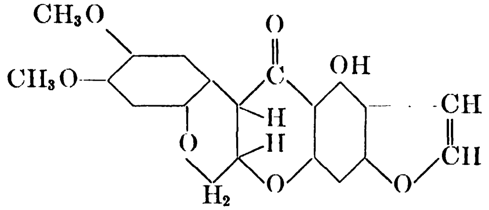
From the concentrated ether extract of a sample of Sumatra-type derris containing no rotenone and 23.7 per cent of total extractives, Meyer and Koolhaas (60) obtained a small amount of a yellowish solid material. On purification this material yielded greenish yellow needle-shaped crystals, which were optically active. The same compound was also obtained a short time later by Harper (39) from the same type of derris.

From its analysis, which indicates the molecular formula $C_{20}H_{16}O_7$, and from the fact that, like toxicarol, it gives a green color with alcoholic ferric chloride, Meyer and Koolhaas (60) proposed the structural formula XXVI for malaccol.



XXVI
Malaccol

After a detailed study of the compound Harper (39) independently proposed the same formula. He found that malaccol gives a positive Durham test, and has two methoxyl groups and an active carbonyl group, as shown by the formation of an oxime. These facts indicate that rings A, B, and C are the same as in rotenone. The strong ferric chloride reaction indicates that there is a phenolic hydroxyl group in the position ortho to the carbonyl group. In agreement with this conclusion it has been observed that malaccol, like sumatrol, is almost insoluble in aqueous alkali. That ring D is a phloroglucinol residue is shown by the close similarity of the ferric chloride colors given by malaccol and its dihydro and tetrahydro derivatives to those of the corresponding derivatives of sumatrol, in which the presence of a phloroglucinol residue has recently been confirmed by synthesis. Harper (39) points out, however, that the experimental data do not distinguish between the angular formula (XXVI) and the less likely linear formula (XXVII). Formula XXVI bears the same structural relation to elliptone as do sumatrol to rotenone and toxicarol to deguelin.



XXVII
Malaccol (linear formula)

G. *Elliptone*

Several years ago Buckley (5) found that an ethereal solution of *Derris elliptica* resin that had been repeatedly treated with 5 per cent potassium hydroxide solution deposited crystals on standing. The purified compound, called at first Buckley's compound and subsequently elliptone[6] (37), was optically inactive, and the suggestion was therefore made that the compound probably did not occur in the resin as such. This surmise proved correct, for a short time later (36, 37, 60) an optically active isomer that could be racemized to *dl*-elliptone was obtained. Both compounds yielded the same dehydro compound, thus confirming further their relationship. The speculation of Boam and Cahn (2) that elliptone is a degradation product of deguelin was therefore untenable. Preliminary insecticidal tests (61) indicate that elliptone is comparable with rotenone in its toxicity to caterpillars.

The molecular formula $C_{20}H_{16}O_6$ (36, 37, 60) was established for elliptone. It shows color tests characteristic of rotenone and closely related compounds, forms an oxime and a dehydro compound, and gives the degradation products obtainable from related compounds having a chromane–chromanone ring system. Although the evidence presented makes the proposed structural formula XXVIII probable, final proof can be afforded only by degradation to compounds of known structure or by synthesis.

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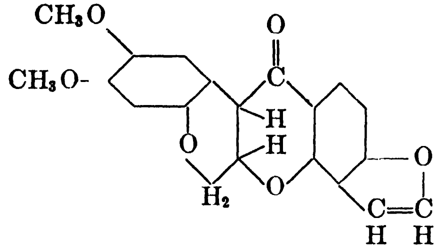
[6] The term "derride" proposed for this substance has already been used by Greshoff (31) for a resinous extract from *Derris elliptica* root.



XXVIII
Elliptone

H. Other constituents

By steam distillation of an extract of the leaves of *Tephrosia vogelii*, Hanriot (35) isolated an oily liquid of the formula $C_{10}H_{16}O$. The compound had the reducing properties of an aldehyde, but no definite derivatives could be prepared. The non-crystalline part of the extractives from *T. virginiana* was investigated by Goodhue and Haller (29), and was found to contain a considerable amount of an oil composed mostly of sesquiterpenes. Two crystalline compounds were isolated by a process including adsorption on charcoal and molecular distillation. One of these, melting at 131°C. and having the molecular formula $C_{21}H_{22}O_4$, had previously been isolated by Clark (21). The other was an orange-yellow crystalline compound melting at 125°C. Analyses indicate the molecular formula to be $C_{22}H_{24}O_4$. Neither of these compounds showed any toxicity to houseflies (30).

TABLE 1

Relative toxicity to insects of rotenone and allied substances

SUBSTANCE	APPROXIMATE RELATIVE TOXICITY	TEST INSECT	REFERENCE
Rotenone	100		
Deguelin (inactive)	10	Bean aphid (*Aphis rumicis* L.)	(23)
	10	Housefly (*Musca domestica* L.)	(70)
	30	Silkworm (*Bombyx mori* L.)	(68)
Deguelin concentrate (active)	50	Housefly (*Musca domestica* L.)	(70)
Tephrosin (inactive)	2	Bean aphid (*Aphis rumicis* L.)	(23)
	10	Silkworm (*Bombyx mori* L.)	(68)
Toxicarol (inactive)	<1	Bean aphid (*Aphis rumicis* L.)	(23)
Toxicarol (active)	7	Bean aphid (*Aphis rumicis* L.)	(76)
Sumatrol (active)	7	Bean aphid (*Aphis rumicis* L.)	(76)
Dihydrorotenone (active)	70	Housefly (*Musca domestica* L.)	(70)
	>30	Silkworm (*Bombyx mori* L.)	(68)
Dihydrodeguelin (inactive)	<3	Housefly (*Musca domestica* L.)	(70)
Dihydrodeguelin (active)	50	Housefly (*Musca domestica* L.)	(70)
Dehydrorotenone (active)	0	Imported cabbage worm (*Ascia rapae* (L.))	(24)

A crystalline material of an acidic nature has been isolated from an unknown species of *Lonchocarpus* by Jones (42). The substance, melting at 201°C. but probably dimorphic, was called lonchocarpic acid. It had the molecular formula $C_{26}H_{26}O_6$ with one methoxy group.

III. TOXICITY TO INSECTS OF ROTENONE AND RELATED COMPOUNDS

Many toxicity tests have been made of the compounds discussed in this paper. In table 1 a brief summation is given of the approximate relative toxicities, on the basis of rotenone as 100, of some constituents of derris and cube and their derivatives. All substances can be compared with rotenone, but direct comparison of the compounds with each other is not entirely valid, as all were not tested in the same way or against the same insect. Comparisons are further complicated by the use of different criteria for toxicity.

REFERENCES

(1) ANONYMOUS: Bull. Imp. Inst. **13**, 61 (1915).
(2) BOAM, J. J., AND CAHN, R. S.: J. Chem. Soc. **1938**, 1818.
(3) BOAM, J. J., CAHN, R. S., AND STUART, A.: J. Soc. Chem. Ind. **56**, 91T (1937).
(4) BRIDGE, W., HEYES, R. G., AND ROBERTSON, A.: J. Chem. Soc. **1937**, 279.
(5) BUCKLEY, T. A.: J. Soc. Chem. Ind. **55**, 285T (1936); Chemistry & Industry **57**, (45), 1059 (1938).
(6) BUTENANDT, A.: Ann. **464**, 253 (1928).
(7) BUTENANDT, A., AND HILGETAG, G.: Ann. **495**, 172 (1932); **506**, 158 (1933).
(8) BUTENANDT, A., AND MCCARTNEY, W.: Ann. **494**, 17 (1932).
(9) CAHN, R. S., AND BOAM, J. J.: J. Soc. Chem. Ind. **54**, 37T (1935).
(10) CAHN, R. S., AND BOAM, J. J.: J. Soc. Chem. Ind. **54**, 42T (1935).
(11) CAHN, R. S., PHIPERS, R. F., AND BOAM, J. J.: J. Chem. Soc. **1938**, 513, 734.
(12) CAHN, R. S., PHIPERS, R. F., AND BOAM, J. J.: J. Soc. Chem. Ind. **57**, 200 (1938).
(13) CAMPBELL, F. L., SULLIVAN, W. N., AND JONES, H. A.: Soap **10** (3), 81 (1934).
(14) CLARK, E. P. Science **71**, 396 (1930).
(15) CLARK, E. P. J. Am. Chem. Soc. **52**, 2461 (1930).
(16) CLARK, E. P. J. Am. Chem. Soc. **53**, 313 (1931).
(17) CLARK, E. P. J. Am. Chem. Soc. **53**, 729 (1931).
(18) CLARK, E. P. J. Am. Chem. Soc. **53**, 2007 (1931); **54**, 3000 (1932).
(19) CLARK, E. P. J. Am. Chem. Soc. **53**, 2264 (1931).
(20) CLARK, E. P. J. Am. Chem. Soc. **54**, 2537 (1932).
(21) CLARK, E. P. Science **77**, 311 (1933).
(22) CLARK, E. P., AND CLABORN, H. V.: J. Am. Chem. Soc. **54**, 4454 (1932).
(23) DAVIDSON, W. M.: J. Econ. Entomol. **23**, 877 (1930).
(24) DAVIDSON, W. M., AND JONES, H. A.: J. Econ. Entomol. **24**, 257 (1931).
(25) FINK, D. E., AND HALLER, H. L.: J. Econ. Entomol. **29**, 594 (1936).
(26) GEORGE, S. W., ROBERTSON, A., SUBRAMANIAM, T. S., AND HILTON, W.: J. Chem. Soc. **1937**, 1535.
(27) GOODHUE, L. D., AND HALLER, H. L.: J. Am. Chem. Soc. **61**, 486 (1939).
(28) GOODHUE, L. D., AND HALLER, H. L.: Ind. Eng. Chem., Anal. Ed. **11**, 640 (1939).
(29) GOODHUE, L. D., AND HALLER, H. L.: J. Am. Chem. Soc. **62**, 2520 (1940).
(30) GOODHUE, L. D., AND SULLIVAN, W. N.: J. Econ. Entomol. **34**, 77 (1941).
(31) GRESHOFF, M.: Ber. **23**, 3538 (1890).
(32) HAAG, H. B.: J. Pharmacol. **43**, 193 (1931).
(33) HALLER, H. L.: J. Am. Chem. Soc. **54**, 2126 (1932).
(34) HALLER, H. L., AND LAFORGE, F. B.: J. Am. Chem. Soc. **56**, 2415 (1934).
(35) HANRIOT, M.: Compt. rend. **144**, 150, 190 (1907).

(36) Harper, S. H.: J. Chem. Soc. **1939,** 1099.
(37) Harper, S. H.: Chemistry & Industry **58,** 292 (1939).
(38) Harper, S. H.: J. Chem. Soc. **1939,** 812.
(39) Harper, S. H.: J. Chem. Soc. **1940,** 309.
(40) Holman, H. J.: *A Survey of Insecticide Materials of Vegetable Origin*, p. 50. Imperial Institute, London (1940).
(41) Jones, H. A.: J. Am. Chem. Soc. **53,** 2738 (1931).
(42) Jones, H. A.: J. Am. Chem. Soc. **56,** 1247 (1934).
(43) Jones, H. A., Campbell, F. L., and Sullivan, W. N.: Soap **11** (9), 99 (1935).
(44) Jones, H. A., Gersdorff, W. A., Gooden, E. L., Campbell, F. L., and Sullivan, W. N.: J. Econ. Entomol. **26,** 451 (1933).
(45) Jones, H. A., and Smith, C. M.: J. Am. Chem. Soc. **52,** 2554 (1930).
(46) Jones, H. A., and Wood, J. W.: J. Am. Chem. Soc. **63,** 1760 (1941).
(47) Kenny, T. S., Robertson, A., and George, S. W.: J. Chem. Soc. **1939,** 1601.
(48) King, H.: Annual Reports of the Chemical Society **29,** 186 (1932).
(49) LaForge, F. B., and Haller, H. L.: J. Am. Chem. Soc. **56,** 1620 (1934).
(50) LaForge. F. B., Haller, H. L., and Smith, L. E.: Chem. Rev. **12,** 182 (1933).
(51) LaForge, F. B., and Markwood, L. N.: Ann. Rev. Biochem. **7,** 473 (1938).
(52) LaForge, F. B., and Smith, L. E.: J. Am. Chem. Soc. **51,** 2574 (1929).
(53) LaForge, F. B., and Smith, L. E.: J. Am. Chem. Soc. **52,** 1091 (1930).
(54) LaForge, F. B., and Smith, L. E.: J. Am. Chem. Soc. **52,** 2878 (1930).
(55) Little, V. A.: J. Econ. Entomol. **24,** 743 (1931); **28,** 707 (1935).
(56) Martin, J. T.: Ann. Applied Biol. **27,** 274 (1940).
(57) McIndoo, N. E., Sievers, A. F., and Abbott, W. S.: J. Agr. Research **17,** 177 (1919).
(58) Merz, K. W.: Arch. Pharm. **270,** 362 (1932).
(59) Merz, K. W., and Schmidt, G.: Arch. Pharm. **273,** 1 (1935).
(60) Meyer, T. M., and Koolhaas, D. R.: Rec. trav. chim. **58,** 207 (1939).
(61) Meyer, T. M., and Koolhaas, D. R.: Rec. trav. chim. **58,** 875 (1939).
(62) Nagai, K.: J. Chem. Soc. Japan **23,** 740 (1902).
(63) Roark, R. C.: J. Econ. Entomol. **24,** 328 (1931).
(64) Roark, R. C.: U. S. Dept. Agr. Misc. Pub. 120 (1934); U. S. Dept. Agr. Bur. Entomol. Plant Quarantine, E-402 (1937), E-453 (1938) (mimeographed).
(65) Roark, R. C.: J. Econ. Entomol. **34,** 684 (1941).
(66) Robertson, A., and Rusby, G. L.: J. Chem. Soc. **1937,** 497.
(67) Rowaan, P. A., and Duuren, A. J. van: Chem. Weekblad **35,** 755 (1938).
(68) Shepard, H. H., and Campbell, F. L.: J. Econ. Entomol. **25,** 142 (1932).
(69) Sievers, A. F., Russell, G. A., Lowman, M. S., Fowler, E. D., Erlanson, C. O., and Little, V. A.: U. S. Dept. Agr. Tech. Bull. 595 (1938).
(70) Sullivan, W. N., Goodhue, L. D., and Haller, H. L.: Soap **15** (7), 107 (1939).
(71) Takei, S., Miyajima, S., and Ono, M.: Ber. **66,** 1826 (1933).
(72) Tattersfield, F., Gimingham, C. T., and Morris, H. M.: Ann. Applied Biol. **13,** 424 (1926).
(73) Tattersfield, F., and Martin, J. T.: Ann. Applied Biol. **22,** 578 (1935).
(74) Tattersfield, F., and Martin, J. T.: Ann. Applied Biol. **23,** 880 (1936).
(75) Tattersfield, F., and Martin, J. T.: J. Soc. Chem. Ind. **56,** 77T (1937).
(76) Tattersfield, F., and Martin, J. T.: Ann. Applied Biol. **25,** 411 (1938).
(77) Tischler, N.: J. Econ. Entomol. **28,** 215 (1935).

STRUCTURE AND ANTIPNEUMOCOCCIC ACTIVITY IN THE CINCHONA SERIES

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The present survey gives particular emphasis to recent studies of the antipneumococcic action of some cinchona derivatives. A brief historical survey is presented and reference is made to speculations concerning the mode of action of cinchonas in the animal body. Antipneumococcic action is analyzed with regard to isomeric and structural modifications of the cinchona molecule. A summary of recent data on bacteriostasis, toxicity, animal protection, pharmacologic testing, and clinical studies is given. Synthetic analogs are discussed in relation to significant structural factors.

Cinchona alkaloids have been used as antimalarials since 1630, but evidence of the specific antipneumococcic action of these compounds dates only from 1911. Morgenroth's (84) study of cinchonas as antipneumococcic agents has been described as "the first striking instance of the successful application of a chemical to the treatment of a bacterial infection in a laboratory animal" (53). Because Morgenroth had been studying quinine in relation to trypanosome infections in animals and was acquainted with the capsule-formation characteristic of trypanosomes, spirilla, and the pneumococcus, he was led to investigate the action of the alkaloid in pneumococcic septicemia in mice. There followed an extensive study of hydrocupreine ethers with the synthesis of the well-known optochin, vuzin, and eucupin. Between 1919 and 1924 Heidelberger and Jacobs (42, 54) at the Rockefeller Institute and Giemsa (32, 33) in Germany published the results of further studies in the hydrocupreine series. These results have been reviewed by von Oettingen (111) and by Houben (50), and the clinical findings have been discussed by Moore and Chesney (87), by Kolmer (63), and by Solis-Cohen (107).

The remarkable antipneumococcic action of optochin has been a spur to reinvestigation of this field. In again studying cinchona derivatives the search was not so much for a compound of greater power but rather for a compound free from deleterious side-effects. The last decade has brought extensive synthesis in the field of apocinchonas. In the early 1930's methylapocupreine[1] [or isoquinine (39)] and ethylapocupreine[1] or [ethylapoquinine (13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90)] were reported by investigators in the United States, Germany, and Japan to have protective action in experimental pneumonias. Since 1934 Maclachlan *et al.* have published results of the experimental testing of alkyl and hydroxylated alkyl ethers of apocupreine in lower animals and of the use of hydroxyethylapocupreine in the treatment of pneumonia in man. In clinical use, this latter compound has been very effective and not a single instance of eye damage has been encountered. The recent Japanese work has consisted largely of experimental studies of antipneumococcic action, and the English investigators have reported antimalarial studies of apocinchonas and similar compounds.

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[1] At the time the term apocupreine (8) was suggested, its use was based firstly on the similarities of apocupreine (formula II) and cupreine (formula I), which differ chemically only in the 3-substituent of ethylidene or vinyl, respectively, and secondly on the established usage of Léger (59, 66) in applying the terms apocinchonine and apocinchonidine (characterized by the ethylidene side chain) to the corresponding substances derived from cinchonine and cinchonidine. In the case of quinine, demethylation is accomplished by boiling with 60 per cent sulfuric acid; a shift of the double bond also occurs. The product, called apoquinine by Hesse (49), consists of substances of different toxicities and antipneumococcic powers and these give derivatives of various biological properties. The name

Hegner (41) has reported hydroxyethylapocupreine and quinine as approximately equally effective against *P. lophurae* in ducks, *P. relictum* in pigeons, and *P. cathemerium* in canaries. The low toxicity of hydroxyethylapocupreine gives particular interest to these findings.

Morgenroth (84) pointed out the importance of systematic variation of the component groups in the cinchona molecule in relation to an ultimate understanding of the mode of antimalarial action or the basis of bacterial specificity of this class of alkaloids. In attempting to correlate chemical structure and bactericidal power the results of the experimental testing of a large number of compounds are considered. It must be remembered that comparison of the data from different workers involves many variables: differences in type and virulence of organism, method of determination of power *in vitro*, age and sex of animals used, method of inducing infection and of administration of the drug, —whether oral, intravenous, or intraperitoneal. Even seasonal factors may influence the numerical results. Marshall (75) has cited the difficulties and has contributed in great measure to the standardization of such biological studies. In the cinchona series a general relationship between the bacteriostatic action *in vitro* and the protective action *in vivo* seems to exist (25, page 771; 83a; 10). Hence the preliminary testing of the antipneumococcic action of this type of drug has included three determinations: bacteriostasis *in vitro*, toxicity to mice, and protective action against pneumococcal septicemia in mice.

From the chemical point of view the cinchona molecule is a comparatively large and complex one and is susceptible to many types of modification. Nature has provided but few of the many possible variants. The best-known naturally occurring alkaloids are the stereoisomeric 6′-methoxy compounds, quinine and quinidine, and the corresponding methoxyl-free compounds, cinchonidine and cinchonine. These have little antipneumococcic action (73, 10).

Formula I represents cupreine, and the corresponding methyl ether is quinine. The quinoline and quinuclidine nuclei linked through the secondary alcoholic

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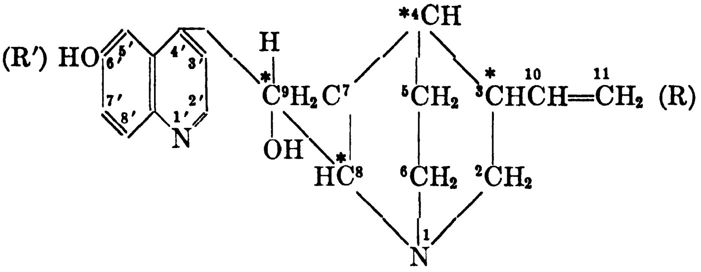
apocupreine has been applied to the main component of the reaction product (13) and corresponds to the terms apoquinine (45, 46), as used by Henry, and β-isocupreine (55), as used by Suszko.



I
Cupreine

group are characteristic of the naturally occurring alkaloids (quinine, quinidine, cupreine, cinchonidine, and cinchonine, and their dihydro derivatives). It is perhaps permissible to consider this rubanol nucleus of quinoline and quinuclidine joined through the secondary alcohol as the power center (contributing structural arrangement, electronic energies, basicity, oxidation potential, or some such fundamental function) and the substituent groups of R at position 3 in the quinuclidine ring and R′ at 6′ in the quinoline ring as having directive influences. At least, any marked change in the rubanol structure usually lessens or destroys bactericidal action in general, whereas changes in R and R′ often serve to modify toxicity and bacterial specificity. The properties of recent synthetic analogs of quinine have emphasized some of the limits in the simplification of structure and have indicated some promising modifications (1, 19, 31, 61).

Among the general factors which have been variously emphasized in considering the mode of action of cinchonas are molecular weight (61, 106, 114), greater effectiveness at a somewhat alkaline pH (62, 78), and surface tension, which is lower in neutral or alkaline solution (17, 78). Ostromislensky (93) stressed the importance of the colloidal nature of a medicinal and thought that the quinoline residue gives colloidal properties and bacterial specificity. Domagk (24) considers the biologically functioning group to be the quinuclidine nucleus, or the nitrogen of this nucleus. Henry (43, page 441) and Findlay (26, page 115) call attention to the decrease in antimalarial action in the case of changes which lessen the basicity of the molecule. The correlation of fluorescence with the antimalarial action of the cinchonas and of atabrin has been noted by Oesterlin (92). Berkenheim (3) has suggested an electronic interpretation of the action of quinoline derivatives.

The mechanism of the action against the pneumococcus is not understood, but this action must differ from that of members of the sulfanilamide group. In contrast with the effect of sulfonamides, the typing characteristics of the organism are not altered when the cinchonas are used in treating clinical cases of pneumonia; no observable change occurs in the bacterial capsule during studies *in vitro*. An interesting observation by Felton and Dougherty (25, page 771) was a so-called "therapeutic zone phenomenon" in the treatment of pneumococcic septicemia in mice. In the range of non-toxic concentrations of the cinchona there was an optimum dose for protective action, and with the use of an amount larger or smaller than the optimum, mice died of septicemia.

A study of cinchonas in relation to biological activity must consider both isomeric and structural modifications. The following main headings with some subdivisions have been used:

I. Stereoisomerism
II. Variations in the quinoline nucleus
    A. Simple substituted quinolines
    B. Modifications of the 6′-substituent (R′) in quinoline, as H, HO, RO in the cinchonas
    C. Substitution in the quinoline ring of the cinchona: nitro, amino, and azo derivatives
    D. Reduction of the quinoline nucleus
III. Variations of the secondary alcohol bridge
IV. Variations in the quinuclidine nucleus
    A. Modification of the 3-substituent (R)
    B. Quaternary salts
    C. Nitrogen-oxide derivatives
    D. Fission of the quinuclidine ring at the 1,8-position (cupreicine derivatives)
        Fission at the 1,2-position (niquine, niquidine, cinchonhydrines)
V. Detailed study of variations in the ether grouping
VI. Synthetic analogs

I. STEREOISOMERISM

Bactericidal action and physiological properties are found to be markedly influenced by the optical configuration of the asymmetric carbon atoms. For instance, some individuals are allergic to quinine but can be successfully treated for malaria with quinidine without unpleasant side reactions, as shown by Dawson and Newman (23) and by Sanders (103). Dawson and Garbade (23) in studying an individual with a quinine idiosyncrasy, demonstrable by Boerner's test, found the subject allergic to the levorotatory drugs quinine, dihydroquinine, cinchonidine, dihydrocinchonidine, ethyldihydrocupreine (optochin), dihydrocupreine, and ethylquitenine but not allergic to the dextrorotatory isomers quinidine, dihydroquinidine, cinchonine, dihydrocinchonine, ethyldihydrocupreidine, dihydrocupreidine, or ethylquitenidine.

The asymmetric centers in formula I are indicated by asterisks on carbon atoms 3, 4, 8, and 9. (The numbering introduced by Rabe (97) has been adopted in most publications, although it is not in strict conformity with the rules established by the International Commission (94)). C_4 has a levo rotation, as observed by Solomon (108a) in methylapocupreicine and in other apocupreicine ethers (99), and the combined rotational value of carbon atoms 3 and 4, in the naturally occurring cinchona alkaloids, indicates a dextro configuration for carbon atom 3 (43, page 423). The steric arrangement of carbon atoms 8 and 9 in the four epimeric modifications was established by Rabe (97) and is shown below:

QUININE CINCHONIDINE		QUINIDINE CINCHONINE		EPIQUININE EPICINCHONIDINE		EPIQUINIDINE EPICINCHONINE	
9	8	9	8	9	8	9	8
−	−	+	+	+	−	−	+

For the typical cinchona structure, the levorotatory quinine derivatives are more effective than the dextro isomers against both the malarial parasite and the pneumococcus, as evidenced by dihydroquinine and optochin in contrast with the dextro forms. The comparative antimalarial values of the dextro and levo series of different types of ethers have been presented by Buttle (14). Where an asymmetric center has been modified in structure, no predictions can be made concerning the more effective optical isomer. Both in the "apo" series (formula II) with the double bond at C_3 and in the niquine series where the quinuclidine nucleus has been changed as shown in formula V, the dextro forms show somewhat greater antimalarial action than the corresponding levo forms; the antipneumococcic power of these derivatives in comparison with the levo isomers is not known.

Epimerization at C_8 and C_9 takes place when a naturally occurring alkaloid such as quinine is refluxed with potassium hydroxide in amyl alcohol and the four theoretically possible isomers are obtained: quinine, quinidine, epiquinine, and epiquinidine. Of these four, epiquinidine is the most effective against the pneumococcus, although all are low in power (73). Another asymmetric center is C_{10} when unsymmetrical additions have been made to the double bond of the vinyl side chain; there is little difference in the antimalarial power of the α-isomer as compared with the β-isomer of chlorodihydroquinine (35) or in the antipneumococcic power of the α- and β-hydroxydihydroquinines (10).

II. VARIATIONS IN THE QUINOLINE NUCLEUS

A. Simple substituted quinolines

It should be noted that some antipneumococcic action can be demonstrated for quinolines, even for those with structures much simpler than the cinchona structure.

In the action of simple quinolines on the pneumococcus, 8-hydroxyquinoline (quinisol) has definite antipneumococcic power but considerable toxicity (11, 111). However, the ethyl, propyl, and hydroxylated ethyl and propyl ethers of 8-hydroxyquinoline had no significant antipneumococcal action (11) and 6-hydroxy or 6-ethoxy derivatives of 2-hydroxyquinolines were ineffective.[2] Bührmann (4) claimed antipneumococcic action at 1:10,000 for a number of quinolines, such as the propyl ester of 6-hydroxyquinoline-4-carboxylic acid or isobutyl 2-phenyl-4-quinolylaminoacetate. In a study of substituted 4-aminoquinolines, Iensch (51) reported a benzthiazole derivative which had prophylactic action in experimental mouse pneumonias but failed in clinical testing.

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[2] Unpublished work.

Attempts to combine the sulfanilamide molecule and an aminoquinoline have not been productive (88, 99) in the simple derivatives, but in the testing of N^4-alkylaminoacetylsulfanilamidoquinolines Juneja (58) has claimed results "comparing favorably with sulfapyridine."

Synthetic derivatives of 6-methoxyquinoline in which a pyrryl (60) or a piperidyl (1) nucleus or a β-dialkylaminomethyl group (61) has been substituted for the quinuclidine portion of the cinchonas have shown action on paramecia or antimalarial power, but no study of the antipneumococcic action has been reported. The preparation and testing of such relatively simple types of synthetic compounds opens an interesting field for future research.

B. Modifications of the 6′-substituent (R′) in quinoline

In the study of antimalarials and in testing the bacteriostatic action of compounds against pneumococcus, streptococcus, or staphylococcus, the 6′-substituent in quinoline has been extensively varied. R′ may be hydrogen, a phenolic hydroxyl, or an ether group. Cinchonas with an unsubstituted hydrogen in the 6′-position have little antipneumococcic value (73).

Phenolic derivatives are obtained when the 6′-methoxydihydrocinchonas undergo demethylation with boiling halogen acids or with 60 per cent sulfuric acid. In the vinyl series a shift of the double bond toward the quinuclidine nucleus takes place, so that the resulting phenolic substances have the ethylidene side chain of the apocinchonas (formula II). Apocupreine had no therapeutic action in cases of clinical pneumonia (72).

In the alkylation of the phenolic cinchonas alkyl, alkoxyalkyl, aroxyalkyl, hydroxyalkyl, and (alkylamino)alkyl ethers have been studied. The remarkable antipneumococcic specificity of ethyldihydrocupreine (optochin) marks a maximal point in the dihydrocupreine series (83a). However, the ethoxyl group is not the only factor necessary, as shown by the fact that the dextrorotatory isomer ethyldihydrocupreidine (prepared by Heidelberger and Jacobs (42a)) had little antipneumococcic action (10), and that ethylcupreine (with the vinyl side chain) was only one-fourth as effective as optochin (50, page 1003). High antipneumococcic action does mark ethylapocupreine (with an ethylidene side chain) (13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90).

The 2-carbon and 3-carbon ethers of hydrocupreine and apocupreine have been the most effective against the pneumococcus, as will be apparent in later tables; higher homologous ethers showed a decreasing antipneumococcic power but indicated an optimal specificity against other pathogens in relation to the length of the ether chain. Because a rather extensive and systematic study has been made of variations of the ether grouping, the more detailed discussion is presented later.

C. Substitution in the quinoline ring of the cinchona: nitro, amino, and azo derivatives

The quinoline nucleus of cinchonas should be subject to typical substitution reactions in the aromatic nucleus. The only modification of this sort as yet reported to give increased bactericidal power has been the introduction of aromatic azo groups, and most of these compounds have marked toxicity. Heidelberger and Jacobs prepared the 5′-nitro- and 5′-amino derivatives of dihydroquinine, optochin, and dihydroquinidine. Using 5′-aminodihydroquinine as a starting point, these investigators obtained 5′-amino-8′-phenylazo-, 5′-amino-8′-(*p*-sulfophenylazo)-, and 5′-hydroxy-8′-phenylazodihydroquinines (54c) as well as 5′, 8′-diamino derivatives (54d). Both 5′-amino-8′-phenylazo-optochin and its dextro isomer were prepared. In coupling diazotized aromatic amines with the phenolic dihydrocupreine and dihydrocupreidine these investigators obtained cinchona dyestuffs of which "many were highly bactericidal *in vitro*" (42b). Boyd found these azo dye derivatives toxic for canaries (5); he reports studies of toxicity, hemolytic action, and antimalarial action for a number of cinchona derivatives. Giemsa and Halberkann reported similar coupling reactions of aromatic diazo compounds with cupreine and dihydrocupreine (32) and in 1933 presented an extensive chemical and chemotherapeutic report on compounds prepared and tested against malaria (33; 26, page 109). Buttle found 5′-sulfamidophenylazoapocupreine (16) of little value against the pneumococcus. At dilutions greater than 1:100,000, neither phenylazoapocupreine nor its ethyl ether showed power against the pneumococcus.[3] Miura found no protective action with small doses of 5′-amino-8′-(sulfamidophenylazo)-ethylhydrocupreine (80). The 5′-amino group of aminodihydroquinine and aminoöptochin has recently been used for the preparation of mustard oils (115); these derivatives have no antimalarial action.

D. Reduction of the quinoline nucleus

Jacobs and Heidelberger (54c) prepared hexahydrocinchonas by reduction of the quinoline ring with sodium and amyl alcohol or with zinc and hydrochloric acid. Boyd (5) found the products toxic and of lower antimalarial power than the unreduced substances.

III. VARIATIONS OF THE SECONDARY ALCOHOL BRIDGE

Many modifications of the secondary alcohol bridge at position 9 have been studied, but in general the changes are dystherapeutic. With regard to antimalarial action, Henry (43, page 443) says that "every alteration so far made in that group has resulted in destruction of antimalarial action," although simple esters such as eucupin are effective *in vivo*. Acetyl and other acyl esters have been made. The Japanese workers found 9-acetylethylapocupreine one-twentieth as effective *in vitro* as ethylapocupreine against the pneumococcus (52), and Maclachlan[4] found that diacetylapocupreine has less inhibiting action on the pneumococcus than apocupreine.

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[3] Unpublished work.

The alcoholic hydroxyl group may be replaced by chlorine, on treatment with phosphorus pentachloride. Chlorine, in turn, may be replaced by hydrogen. Heidelberger and Jacobs studied the 9-chloro and 9-desoxy derivatives of optochin and its dextro isomer, but found these compounds "less bactericidal for the pneumococcus than the corresponding parent alkaloids" (42c). The 9-aminoquinine was prepared by Fränkel (28) and 9-alkylaminoquinines by Altman (2) but were tested only against malaria.

With chromic acid oxidation the alcohol group is converted to a ketone and, surprisingly, Cretcher and Renfrew (100) found that the cinchonas were converted to the corresponding ketones in fair yield during reaction with sodium amide. Dihydroquininone has little action against the pneumococcus (10) and no antimalarial action (43, page 443), but Morgenroth and Bumke (83) claim small antipneumococcic action *in vitro* for 9-ketoöptochin.

Cupreicine derivatives (formula IV) in which 1, 8 rupture of the quinuclidine ring has given 4′-quinolyl β-(4-piperidyl)ethyl ketones are discussed under modifications of the quinuclidine nucleus.

IV. VARIATIONS IN THE QUINUCLIDINE NUCLEUS

A. Modification of the 3-substituent (R)

In relation to antipneumococcic action both 3-ethyl and 3-ethylidene structures are effective. To consider first the saturated side chain:—in the levo series the 3-ethyl substituent is much more effective than the vinyl group, as shown in the comparison of dihydroquinine and quinine (84), and the fact that ethyldihydrocupreine is about four times as effective as ethylcupreine (10, 73; 50, page 1003). The dihydrocinchonas are obtained in limited amount from the cinchona bark and may be readily prepared by reduction of the unsaturated alkaloids. On reduction of the ethylidene group of the apo structures (48), both the known dihydroalkaloid and a new epi-C_3-dihydrocinchona are formed, but no test of such a derived epi-C_3-dihydrocinchona against the pneumococcus has been reported.

Addition of water (10), hydrogen chloride (35), or hydrogen iodide (101, 102) gives rise to C_{10} isomers, as noted in section I. Very low action *in vitro* has been reported for hydroxydihydroquinine (10). Halogen in the side chain slightly lessens the antipneumococcic action, as observed by the Japanese workers (81, 82).

On oxidation of the vinyl group there is loss of the terminal carbon atom and formation of a carboxylic acid. The quitenine structure, ineffective of itself, regains antimalarial power on esterification of the carboxyl, as shown in a series of homologous esters tested by Goodson (36). Ethylquitenine is without power against the pneumococcus (10, 73).

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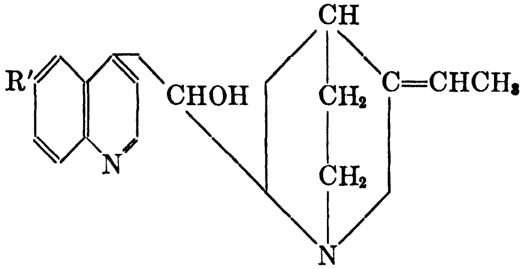
[4] Unpublished work.

The 3-ethylidene group of the apocinchonas (formula II) seems to enhance

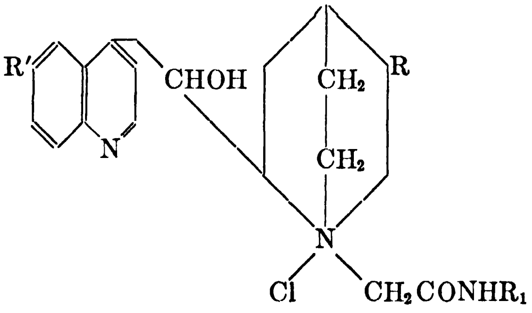


II

antipneumococcic action and to lessen toxicity. Methylapocupreine (isoquinine) is more effective than quinine or dihydroquinine (39, 89; also unpublished work); ethylapocupreine has about the same antipneumococcic action as optochin (13, 80, 89). The apocupreine (apoquinine) ethers have lower toxicity than the corresponding ethers of the hydrocupreine or cupreine series, as is apparent in the data cited by Maclachlan (56, 72, 73), Butler (10, 13), and Gundel and Seitz (39). Conversion of the vinyl group to an ethylidene group introduces the possibility of geometric isomerism. Of the two isomeric series only the ethers of apocupreine (6–13; 22, 38, 56, 68–73, 99, 100; 39, 67; 52, 76, 79, 80, 81, 89, 90) have been tested in pneumonia studies, except for some incomplete reports by the Japanese workers (80).

B. *Quaternary salts*

The lowered bactericidal power of most quaternary salts in this series and of the *N*-oxide derivatives of cinchonas is interpreted as emphasizing the rôle of tertiary nitrogen (86, 91) in the bactericidal action in cinchonas.



III

The rubanol complex has two basic nitrogen atoms which make possible neutral and acid salts (57) and also serve for the formation of quaternary salts. The quinuclidine nitrogen is the first to be converted to the quinquevalent state. Heidelberger and Jacobs studied a great variety of cinchona quaternary salts, including a series of addition products of dihydroquinine with chloroacetanilide, chloroacetylaminophenol, chloroacetyl-*p*-anisidine, chloroacetylaminopyrocatechol, etc. (54a). Although the rather low protective action of dihydroquinine against the pneumococcus is enhanced in the hydroquinine quaternary salts (25, 86), it is apparent from table 1 that the bacteriostatic action against this organism remains at a low level whether the cinchona nucleus of itself is characterized by much or by little antipneumococcic power. *p*-Hydroxyacetanilide cinchonidinium chloride hydrochloride is bacteriostatic at $1:1 \times 10^4$.

Ishizaka (52) found ethylapocupreine bacteriostatic for pneumococcus at 1:20,000, whereas the methochloride and the methoiodide were ineffective at 1:1000; on the other hand, these quaternary salts retained the initial moderate effectiveness of the cinchona against streptococcus and staphylococcus (bacteriostatic at 1:2000).

TABLE 1

Bacteriostatic activity of quaternary compounds of cinchonas against Pneumococcus II

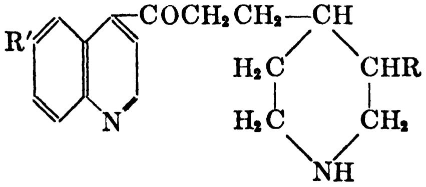
QUATERNARY DERIVATIVE AS A HYDROCHLORIDE	ACTIVE *in vitro* AT CONCENTRATION OF	INVESTIGATOR
p-Chloroacetylaminophenol salt of:		
Dihydroquinine	$1:4 \times 10^4$	Felton (25)
	$1:5 \times 10^4$	Maclachlan *et al.* (18)
Optochin	$1:4 \times 10^4$	Morgenroth (86)
Hydroxyethylapocupreine*	$1:5 \times 10^4$	Maclachlan *et al.* (18)
p-Chloroacetanilide salt of:		
Dihydroquinine	$1:12 \times 10^4$	Felton (25)
	$1:8 \times 10^4$	Morgenroth (86)
Optochin	$1:8 \times 10^4$	Morgenroth (86)
Hydroxyethylapocupreine	$1:1 \times 10^4$	Maclachlan *et al.* (18)

* *p*-Hydroxyacetanilide hydroxyethylapocupreinium chloride hydrochloride.

C. Nitrogen-oxide derivatives

In view of the theories associating bactericidal action with oxidation potential or intermediate oxidation products (105), the *N*-oxides of the cinchonas (formed on the quinuclidine nitrogen) should be of interest. However, the *N*-oxides show less power than the unmodified bases against the malarial parasite (91) or against pneumococci.[5]

D. Fission of the quinuclidine ring at the 1,8-position (cupreicine derivatives)



IV

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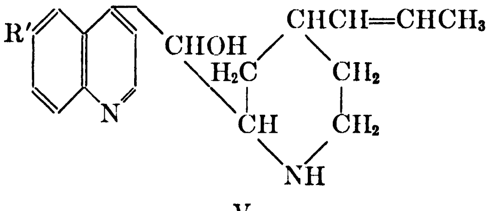
[5] Unpublished work.

Hydrolysis of the 1,8-linkage of the quinuclidine ring leads to compounds of low bacteriostatic power and considerable toxicity (99). Neither reduction of the keto group to give an asymmetric secondary alcohol (42e), nor restoration of a tertiary character to the nitrogen by replacement of the hydrogen of the piperidyl NH group with an alkyl group (42d, 42e) produces active compounds. The introduction of the sulfanilyl radical on the piperidine nitrogen gave compounds without value against the pneumococcus (99).[6] In considering the significance of the 1,8-fission with regard to the correlation of structure and antipneumococcic action, it is important to remember that conversion of the quinuclidine to a piperidine ring is not invariably dystherapeutic, as shown by the antimalarial effectiveness of niquine and niquidine (31, 108b) and of 4′-(6′-methoxyquinolyl)-2-piperidylcarbinol (synthesized by Ainley and King (1)).

Fission of the 1,2-linkage (niquine, niquidine, cinchonhydrines): The fission of the 1,2-linkage to form niquine or niquidine has been formulated clearly only recently. As early as 1890 Skraup observed that when the vinyl side chain of quinine had been saturated by the addition of hydrogen iodide or hydrogen bromide, subsequent removal of the halogen acid gave "isoquinine" (formula II) and niquine (formula V). Léger (66) has studied these transformations especially in the cinchonidine–cinchonine series, where the niquine analogs are called cinchonhydrines; and very recently Gibbs and Henry (31) have shown that the formation of niquine or niquidine involves splitting of the 1,2-linkage with loss of a methylene group as formaldehyde. The resultant structure is shown in formula V.



V

Buttle *et al.* (14) report that niquidine has the highest antimalarial action of any of the *d*-compounds. Although niquine has no effective action against the pneumococcus, nothing is known as yet of the action of the stereoisomers or of the action of higher homologous ethers. Niquidine, like the cupreicines, has a piperidine ring, but the short carbinol bridge to the α-position of this ring is similar to the cinchona linkage.

V. DETAILED STUDY OF VARIATIONS IN THE ETHER GROUPING

Inasmuch as the most useful variations in the bacteriostatic action of the cinchona molecule have been associated with changes of R′ and R, it has seemed desirable to discuss these groups in some detail. Tables 2, 3, and 4 present some of the comparative evaluations of the action *in vitro* and of the toxicity of apocupreine ethers and certain related compounds. To minimize the effect of differences in the methods of obtaining biological data, the numerical values have been taken only from studies made by Maclachlan and his coworkers, but references to other investigators are included and, in general, the findings have been similar.

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[6] Crystalline addition compounds of cinchona alkaloids with sulfanilamide were not more effective than the individual components (109).

TABLE 2

Bacteriostasis against Pneumococcus II

COMPOUND*	FORMULA	CONCENTRATION OF DRUG	REFERENCE
δ-Hydroxy-*n*-butyl-	*n*-HOC_4H_8—	1:0.5 to 2 × 10^5	
β-Hydroxy-*n*-propyl-	$CH_3CHOHCH_2$—		
β-Keto-*n*-propyl-	CH_3COCH_2—		
β-Methyl-β-hydroxy-*n*-propyl-	$(CH_3)_2C(OH)CH_2$—		
Methyl-	CH_3—		
Butoxyethyl-	$C_4H_9OC_2H_4$—		(39)
8-Alkoxyquinolines			
β-Hydroxyethyl-	$HOCH_2CH_2$—	1:3 × 10^5	
β,β′-Dihydroxyisopropyl-	$(HOCH_2)_2CH$—		
Phenoxyethyl-	$C_6H_5OC_2H_4$—		
α-Methyl-β-hydroxy-*n*-propyl-	$CH_3CHOH(CH_3)CH$—	1:4 × 10^5	
α-Methylol-*n*-propyl-	$CH_3CH_2(CH_2OH)CH$—		
n-Butyl-	*n*-C_4H_9—		(14, 67)
Amyl- (four isomers)	C_5H_{11}—		(14, 52, 67, 81, 90)
8-Hydroxyquinoline			(11, 37; 111, p. 33)
Ethyl-	C_2H_5—	1:8 × 10^5	(13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90)
β-Chloroethyl-	ClC_2H_4—		
γ-Hydroxy-*n*-propyl-	HOC_3H_6—		
sec-Butyl-	$CH_3CH_2(CH_3)CH$—		
n-Amyl-	*n*-C_5H_{11}—		(14, 67)
α-Methyl-β-hydroxyethyl-	$HOCH_2(CH_3)CH$—	1:16 × 10^5	
n-Propyl-	*n*-C_3H_7—		(14, 67)
Isopropyl-	$(CH_3)_2CH$—		(14, 52, 67)
Isobutyl-	$(CH_3)_2CHCH_2$—		(52)

* The compounds listed are ethers of apocupreine dihydrochloride except as indicated and are entered according to the maximum dilution effecting complete bacteriostasis *in vitro*.

Bacteriostasis (table 2)

Most of the compounds indicated in table 2 are apocupreine ethers. These compounds are listed according to bacteriostatic action *in vitro*. The dilution of the drug is given as 1 part in $n \times 10^5$. In the cinchona series the protective value of a compound seems roughly to parallel the antipneumococcic action *in vitro*. Both experimental and clinical experience show that the bacteriostatic action is effective against all types of pneumococcus—with more variation with reference to different strains than to difference in type (6, 56).

It is apparent from table 2 that of the ethyl, propyl, butyl, and amyl ethers tested, the greatest antipneumococcic power is associated with 2-carbon and 3-carbon ethers. This corresponds with Morgenroth's finding the maximal action against the pneumococcus shown by ethyldihydrocupreine in the dihydrocupreine series. There is as yet no apparent correlation of effectiveness with the position at which branching occurs in the higher alkyl ether groupings. The apocupreine series thus far prepared is too brief to allow comparison with the antimalarial studies of Buttle *et al.* (14) and Magidson and Strukov (74), who observed a rise and fall of antimalarial action characterizing the *n*-alkyl ethers in separate series of odd- and even-numbered carbon chains. The higher alkyl apocupreine ethers have some action against streptococcus and staphylococcus (52, 81; also unpublished work), and it will be remembered that Morgenroth (85) found the highest bactericidal action for streptococcus in isoöctyldihydrocupreine (vuzin) and for staphylococcus in isoamyldihydrocupreine (eucupin). *In vitro* action of hydroxyethylapocupreine against streptococcus has been reported (113). Ishizaka (52) found isoöctylapocupreine only one-sixteenth as powerful as ethylapocupreine against the pneumococcus.

Modified alkyl ethers were prepared by Slotta and Behnisch (106), who alkylated dihydrocupreine with dialkylaminoalkyl halides to introduce a basic ether group similar to the side chain of plasmoquin and atabrin. Growth of pneumococci was inhibited for 8 hr. by γ-dimethylaminoisoamyldihydrocupreine at a concentration of 1:200,000 with partial inhibition for 24 hr.; other alkylaminoalkyl ethers were less effective. Alkoxyalkyl ethers[7] and aroxyalkyl ethers are represented in the table by butoxyethyl-, phenoxyethyl-, and benzyloxyethyl-apocupreines, which were not found effective against the pneumococcus, although other modifications of the alkyl ether were more successful. Halogen substitution in the alkyl group did not greatly alter the properties of the compound; this is still under investigation. There has not been enough study to allow generalizations with regard to varying the position of the hydroxyl or ketone group.

The study of hydroxyalkyl ethers was undertaken in an attempt so to modify cinchona derivatives that the antipneumococcic power would be retained but host toxicity lessened. When tested in the barbituric acid series, the replacement of alkyl groups by hydroxyalkyl groups had greatly lowered toxicity (21). It seemed a plausible speculation that modification of the hydrocarbon character of the ether groupings of cinchonas might alter the lipoid–water distribution in the direction of water solubility and in this manner avoid the tendency to eye damage which has prevented generalized use of optochin. That hydroxylation did lower animal toxicity is apparent from table 3, in the comparison of hydroxyalkyl ethers with the parent alkyl ethers. The absence of eye damage in dogs receiving massive doses (22) and the complete absence of any eye symptoms in a large group of clinical cases treated with hydroxyethylapocupreine (71) are evidence of the definite modification in physiological properties which may accompany a small change in chemical structure. That this change also brought somewhat lowered antipneumococcic action is evident in the data of table 2.

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[7] The report of a study of alkylthioalkyl apocupreine ethers by Tipson and Cretcher is in press.

Toxicity (tables 3 and 4)

The toxicity data given in table 3 are for intraperitoneal tests. The doses reported as L.D. 50 are milligrams of drug lethal to 50 per cent of the animals (20-g. mice). As indicated for hydroxyethylapocupreine, the L.D. 50 on an oral basis may be six times as large as the intraperitoneal value. It will be noted that the higher homologous alkyl ethers (butyl and amyl) show greater toxicity. The effect of hydroxylation on toxicity and action *in vitro* is summarized in table 4 for the ethyl and isopropyl ethers of apocupreine, to emphasize the remarkable changes in selectivity brought about by slight changes in a large cinchona molecule.

Animal protection

Ethylhydrocupreine, ethylapocupreine, hydroxyethylhydrocupreine, and hydroxyethylapocupreine have protective power when tested in experimental mouse pneumonias (56, 73). β-Isoquinine gave some protection *in vivo* (39), although considerably less than hydroxyethylapocupreine.[8] The action of ethylapocupreine in mouse protection has been reported by various investigators (39, 56, 73, 76, 80, 89); Liebetruth (67) found power *in vivo* noticeably decreased in the higher *n*-alkyl apocupreine ethers. In 1940 Bracken (6) found comparable protection against virulent pneumococci with hydroxyethylapocupreine or sulfapyridine in equal doses; in mouse protection experiments the use of either chemical enhanced the protective action of the other.

Pharmacology

The pharmacology of the cinchonas has been reviewed in considerable detail by von Oettingen (111) and other authors (27, 50, 63, 65, 87, 107). Kruse (64, 69) found that hydroxyethylapocupreine is less toxic to the circulation and respiration of the dog than are quinine or quinidine. Dawson *et al.* (22) reported the ophthalmoscopic examination and histologic study of the eyes of dogs subcutaneously injected with large doses of cinchona ethers. They observed that in massive doses quinine and the alkyl ethers caused a destruction of cells in the ganglionic layer; comparable dosage with hydroxyethylapocupreine caused no demonstrable damage.

Clinical studies

Interesting observations have been made in the course of the clinical study of apocupreine, ethylapocupreine, and hydroxyethylapocupreine. Although in some tests apocupreine had shown bacteriostasis *in vitro* and protective action

[8] Unpublished work.

for mice, it has no therapeutic action in clinical trial. Ethylapocupreine was not acceptable clinically because of temporary eye damage to some individuals; hence other derivatives were sought. The clinical use of hydroxyethylapocupreine in over six hundred cases of pneumococcic pneumonia with good results has been reported by Maclachlan (71) and his associates. The cases included

TABLE 3

Intraperitoneal toxicity

COMPOUND*	APPROXIMATE L.D. 50†
	milligrams
n-C_3H_7—	2–3
C_4H_9— (three isomers)	2–3
C_5H_{11}— (five isomers)	2–3
Ethylapocupreicine	2–3
8-Hydroxyquinoline	2–3
$C_6H_5OC_2H_4$—	2–3
Ethylhydrocupreine	4–5
ClC_2H_4—	4–5
$(CH_3)_2CH$—	4–5
C_2H_5—	4–5
Quinine	5
$(CH_3)_2C(OH)CH_2$—	5+
$CH_3CHOH(CH_3)CH$—	5+
n-HOC_4H_8—	5+
n-HOC_3H_6—	6–8
$CH_3CH_2(CH_2OH)CH$—	6–8
CH_3COCH_2—	6–8
$HOCH_2CH_2$—‡	6–8
$HOCH_2(CH_3)CH$—	6–8
$CH_3CHOHCH_2$—	6–8

* Ethers of apocupreine dihydrochloride except as indicated.

† Dose in milligrams for a 20-g. mouse.

‡ Oral toxicity: L.D. 50 ≈ 40 mg.

TABLE 4

Effect of hydroxylation on toxicity and action in vitro

ETHERS OF APOCUPREINE	TOXICITY APPROX. L.D. 50	BACTERIOSTASIS CONCENTRATION OF DRUG
	milligrams	
C_2H_5—	4	1:8 × 10^5
HOC_2H_4—	7	1:3 × 10^5
$(CH_3)_2CH$—	4	1:16 × 10^5
$HOCH_2CH(CH_3)$—	7	1:16 × 10^5
$(HOCH_2)_2CH$—	9	1:3 × 10^5

many types of pneumococcus (6). The use of the drug does not interfere with the typing of the pneumococcus and no visual disturbance has been observed with any patient; there is "no evidence that the leucocytes are depressed, and ear symptoms do not occur." In direct topical application, hydroxyethylapocupreine monohydrochloride has been found highly effective in treating pneumococcic conjunctivitis (69) and very satisfactory results have been obtained in routine use of an aqueous solution of the dihydrochloride in treating pharyngitis.[9]

VI. SYNTHETIC ANALOGS

In developing methods for the synthesis of aza-bicyclic compounds, Prelog (95) has synthesized the simple quinuclidine. Clemo and Metcalfe (19a) synthesized 3-ketoquinuclidine. No biological testing of these simple units has been reported. Clemo and Hoggarth (19b) found 7-ketoruban to be without antimalarial action, but this compound lacks the secondary alcohol group and the methoxyl substituent in the quinoline nucleus. Both Rabe (98) and Prelog (96) have recently synthesized isomeric 6′-methoxyruban-9-ols, but Rabe reports absence of antimalarial action in any of the four isomers, whereas Prelog reports power similar to quinine in the treatment of bird malaria with the 6′-methoxyrubanol.

That cyclic units other than quinuclidine may serve as the "second half" of cinchona analogs possessing therapeutic properties is apparent from the properties of niquidine (formula V), which is a substituted quinolyl-2-piperidylcarbinol (31, 108b). Ainley and King (1) found that the synthetic 4′-(6′-methoxyquinolyl)-2-piperidylcarbinol had considerable antimalarial action in bird malaria and suggest "that the piperidine ring system should be as near the quinoline ring system as possible, consistent with retention of the carbinol group." The effectiveness of these α-piperidine derivatives is in sharp contrast to the absence of antimalarial power in cupreicine ethers (formula IV). In 1917 Karrer (60) synthesized 4′-(6′-methoxyquinolyl)-2-pyrrylcarbinol and described it as having action of the same order as quinine on paramecia. In studying even simpler compounds which had an ethanol bridge linking 6′-methoxyquinoline with tertiary amines, King and Work (61) found that these structures had small antimalarial power.

CONCLUSION

Thus far the alkoxyquinoline and alkyl-substituted quinuclidine nuclei, bound together by a secondary alcohol bridge, appear to be a necessary basis for antipneumococcic activity in the cinchona series. Maximum activity seems to be shown by the ethyl and isopropyl alkyl ethers. Some types of substitution in the alkyl ether group modify, without wholly changing, the bactericidal and physiological properties. The quinuclidine side chain may be ethyl (e.g., of hydrocupreine) or ethylidene (e.g., of apocupreine); greater antipneumococcic action appears to be associated with the ethylidene group.

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[9] Unpublished work.

The steric configuration of the molecule is a factor, and it is probable that linkage at the α-carbon atom of the quinuclidine nucleus is important.

The fact that certain recently prepared cinchona analogs possess antimalarial action suggests that the quinuclidine nucleus may be replaced by other cyclic nitrogen components. The short secondary alcohol bridge between the two nitrogen units and the spatial arrangement of the linkage at the α-carbon atom of the non-aromatic nitrogen group appear to be important factors in the properties of these compounds.

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REFERENCES

(1) Ainley, A. D., and King, H.: Proc. Roy. Soc. (London) **B125**, 60 (1938).
(2) Altman, R. F. A.: Rec. trav. chim. **57**, 941 (1938).
(3) Berkenheim, A. M.: J. Gen. Chem. (U.S.S.R.) **6**, 1039 (1936); Chem. Abstracts **31**, 1779 (1937).
(4) Bührmann, I.: Z. Immunitäts. **84**, 300 (1935).
(5) Boyd, G. H.: Am. J. Hyg. **6**, 173 (1926).
(6) Bracken, M. M., Johnston, J. M., Crum, G. E., Patrick, D. R., Permar, H. H., and Maclachlan, W. W. G.: J. Pharmacol. **68**, 259 (1940).
(7) Butler, C. L., and Cretcher, L. H.: J. Am. Pharm. Assoc. **22**, 414 (1933).
(8) Butler, C. L., and Cretcher, L. H.: J. Am. Chem. Soc. **57**, 1083 (1935).
(9) Butler, C. L., Hostler, M., and Cretcher, L. H.: J. Am. Chem. Soc. **59**, 2354 (1937).
(10) Butler, C. L., Nelson, W. L., Renfrew, A. G., and Cretcher, L. H.: J. Am. Chem. Soc. **57**, 575 (1935).
(11) Butler, C. L., and Renfrew, A. G.: J. Am. Chem. Soc. **60**, 1473, 1582 (1938).
(12) Butler, C. L., Renfrew, A. G., and Clapp, M. A.: J. Am. Chem. Soc. **60**, 1472 (1938).
(13) Butler, C. L., Renfrew, A. G., Cretcher, L. H., and Souther, B. L.: J. Am. Chem. Soc. **59**, 227 (1937).
(14) Buttle, G. A. H., Henry, I. A., Solomon, W., Trevan, J. W., and Gibbs, E. M.: Biochem. J. **32**, 47 (1938).
(15) Buttle, G. A. H., Henry, T. A., and Trevan, J. W.: Biochem. J. **28**, 426 (1934).
(16) Buttle, G. A. H., Gray, W. H., and Stephenson, D.: Lancet **230**, 1286 (1936).
(17) Chopra, R. N., and Choudhury, S. G.: Indian J. Med. Research **17**, 360 (1929); reference 111, pp. 142, 210.
(18) Clapp, M. A., Renfrew, A. G., and Cretcher, L. H.: J. Am. Chem. Soc. **63**, 2169 (1941).
(19) (a) Clemo, G. R., and Metcalfe, T. P.: J. Chem. Soc. **1937**, 1989.
(b) Clemo, G. R., and Hoggarth, E.: J. Chem. Soc. **1939**, 1241; **1941**, 476.
(20) Cohen, A., and King, H.: Proc. Roy. Soc. (London) **B125**, 49 (1938).
(21) Cretcher, L. H., Koch, J. A., and Pittenger, W. H.: J. Am. Chem. Soc. **47**, 3083 (1925).
Cretcher, L. H., and Pittenger, W. H.: J. Am. Chem. Soc. **46**, 1503 (1924); **47**, 2560 (1925).
(22) Dawson, W. T., Permar, H. H., Johnston, J. M., and Maclachlan, W. W. G.: Am. J. Med. Sci. **193**, 543 (1937).
(23) Dawson, W. T., and Garbade, F. A.: J. Am. Med. Assoc. **94**, 704 (1930).
Dawson, W. T., and Newman, S. P.: J. Am. Med. Assoc. **97**, 930 (1931).
(24) Domagk, G.: Angew. Chem. **48**, 657 (1935).

(25) FELTON, L. D., AND DOUGHERTY, K. M.: J. Exptl. Med. **35**, 761 (1922); **36**, 163 (1922).
(26) FINDLAY, G. M.: *Recent Advances in Chemotherapy.* The Blakiston Company, Philadelphia, Pennsylvania (1939).
(27) FISCHL, V., AND SCHLOSSBERGER, H.: *Handbook of Chemotherapy*, English translation. H. G. Roebuck and Son, Baltimore (1933).
(28) FRÄNKEL, S., TRITT, C., MEHRER, M., AND HERSCHMANN, O.: Ber. **58**, 544 (1925).
(29) GANAPATHI, K.: Indian J. Med. Research **27**, 947 (1940).
(30) GHOSH, S., AND CHATTERJEE, W. R.: J. Indian Chem. Soc. **8**, 257 (1931).
(31) GIBBS, E. M., AND HENRY, T. A.: J. Chem. Soc. **1939**, 240, 1294.
(32) GIEMSA, G., AND HALBERKANN, J.: Ber. **52**, 906 (1919).
(33) GIEMSA, G., AND OESTERLIN, M.: Arch. Schiffs-Tropen-Hyg. **37**, 217 (1933).
(34) GOODMAN, L., AND GILMAN, A.: *The Pharmacological Basis of Therapeutics.* The Macmillan Company, New York (1941).
(35) GOODSON, J. A.: J. Chem. Soc. **1935**, 1094.
(36) GOODSON, J. A., HENRY, T. A., AND MACFIE, J. W. S.: Biochem. J. **24**, 874 (1930).
(37) GRAY, W. H.: J. Chem. Soc. **1939**, 1202.
(38) GREEN, M. H., RENFREW, A. G., AND BUTLER, C. L.: J. Am. Chem. Soc. **61**, 1783 (1939).
(39) GUNDEL, M., AND SEITZ, L.: Z. Immunitäts. **80**, 240 (1933).
(40) HEFFRON, R.: *Pneumonia.* The Commonwealth Fund, New York (1939).
(41) HEGNER, R., WEST, E., RAY, M., AND DOBLER, M.: Am. J. Hyg. **33**, 101 (1941). HEGNER, R., WEST, E., AND DOBLER, M.: Am. J. Hyg. **34**, 132 (1941).
(42) HEIDELBERGER, M., AND JACOBS, W. A.: J. Am. Chem. Soc. (a) **41**, 817 (1919); (b) **41**, 2131 (1919); (c) **42**, 1489 (1920); (d) **44**, 1091 (1922); (e) **44**, 1098 (1922).
(43) HENRY, T. A.: *Plant Alkaloids*, 3rd edition. The Blakiston Company, Philadelphia, Pennsylvania (1939).
(44) HENRY, T. A.: Chemistry & Industry **55**, 111 (1936).
(45) HENRY, T. A., AND SOLOMON, W.: J. Chem. Soc. **1934**, 1923.
(46) HENRY, T. A., AND SOLOMON, W.: Chemistry & Industry **54**, 641 (1935).
(47) HENRY, T. A., SOLOMON, W., AND GIBBS, E. M.: J. Chem. Soc. **1935**, 966.
(48) HENRY, T. A., SOLOMON, W., AND GIBBS, E. M.: J. Chem. Soc. **1937**, 592.
(49) HESSE, O.: Ann. **205**, 314 (1880).
(50) HOUBEN, J.: *Fortschritte der Heilstoffchemie*, zweite Abteilung, Band III, Houben und Pfankuch, "Die Heterocyklischen Verbindungen," pp. 744–1033. Walter de Gruyter, Berlin (1939).
(51) IENSCH, H.: Angew. Chem. **50**, 891 (1937).
(52) ISHIZAKA, N., OKAMOTO, H., MIURA, K., MATSUDA, S., AND SHAKO, T.: Japan J. Med. Sci. IV. Pharmacol., Proc. **7**, 42 (1933).
(53) JACOBS, W. A.: *The Harvey Lectures*, 1923–24. J. B. Lippincott, Philadelphia, Pennsylvania.
(54) JACOBS, W. A., AND HEIDELBERGER, M.: J. Am. Chem. Soc. (a) **41**, 2090 (1919); (b) **42**, 1481 (1920); (c) **42**, 2278 (1920); (d) **44**, 1073 (1922); (e) **44**, 1079 (1922).
(55) JARZÝNSKI, L., LUDWICZAKÓWNA, R., AND SUSZKO, J.: Rec. trav. chim. **52**, 839 (1933).
(56) JOHNSTON, J. M., BURCHELL, H. B., PERMAR, H. H., AND MACLACHLAN, W. W. G.: J. Pharmacol. **61**, 364 (1937).
(57) JOHNSON, F. F.: J. Am. Pharm. Assoc. **26**, 1227 (1937).
(58) JUNEJA, G. L., NARANG, K. S., AND RAY, J. N.: J. Indian Chem. Soc. **17**, 495 (1940).
(59) JUNGFLEISCH, E., AND LÉGER, E.: Ann. chim. [9] **14**, 59 (1920).
(60) KARRER, P.: Ber. **50**, 1499 (1917).
(61) KING, H., AND WORK, T. S.: J. Chem. Soc. **1940**, 1307.
(62) KOHLTOFF, J. M.: Biochem. Z. **162**, 289 (1925).
(63) KOLMER, J. A.: *Principles and Practice of Chemotherapy.* W. B. Saunders, Philadelphia, Pennsylvania (1926).
(64) KRUSE, K. T.: J. Pharmacol. **66**, 20 (1939).

(65) Laqueur, E.: *Die neuren chemotherapeutischen Präparate aus der Chininreihe und aus der Akridinreihe.* Julius Springer, Berlin (1923).

(66) Léger, E.: Bull. soc. chim. [4] **23**, 133, 142, 240, 328 (1918).
Léger, E.: J. pharm. chim. [8] **23**, 558 (1936).

(67) Liebetruth, E.: Z. Immunitäts. **84**, 445 (1935).

(68) MacLachlan, W. W. G.: International Clinics IV, 127 (1937); Series 47.

(69) MacLachlan, W. W. G.: Penn. Med. J., February, 1940.

(70) MacLachlan, W. W. G., Johnston, J. M., Bracken, M. M., and Crum, G. E.: Am. J. Med. Sci. **197**, 31 (1939).

(71) MacLachlan, W. W. G., Johnston, J. M., Bracken, M. M., and Pierce, L. S.: Am. J. Med. Sci. **201**, 367 (1941).

(72) MacLachlan, W. W. G., Permar, H. H., Johnston, J. M., and Burchell, H. B.: Am. J. Med. Sci. **194**, 474 (1937).

(73) MacLachlan, W. W. G., Permar, H. H., Johnston, J. M., and Kenney, J. R.: Am. J. Med. Sci. **188**, 699 (1934).

(74) Magidson, O. Yu., and Strukov, I. T.: Arch. Pharm. **271**, 359 569 (1933).

(75) Marshall, E. K.: Bull. N. Y. Acad. Med. **16**, 723 (1940).

(76) Matsuda, S.: Japan J. Med. Sci. IV. Pharmacol., Proc. **7**, 45 (1933); Proc. **8**, 30 (1934).

(77) May, P., and Dyson, G. M.: *The Chemistry of Synthetic Drugs.* Longmans, Green and Company, London (1939).

(78) Michaelis, L., and Dernby, K. G.: Z. Immunitäts. **34**, 194 (1922).

(79) Miura, K.: Japan J. Med. Sci. IV. Pharmacol. **11** (Trans. of the 12th Annual Meeting), 150 (1938).

(80) Miura, K.: Japan J. Med. Sci. IV. Pharmacol. **12**, 209 (1940).

(81) Miura, K., and Okamoto, H.: Japan J. Med. Sci. IV. Pharmacol., Proc. **5**, 1 (1930).

(82) Miura, K., and Sogen, Y.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5**, 41 (1931).

(83) Morgenroth, J., and Bumke, E.: Deut. med. Wochschr. (a) **40**, 538 (1914); (b) **44**, 729 (1918).

(84) (a) Morgenroth, J., and Levy, R.: Berlin klin. Wochschr. **48**, 1560, 1979 (1911).
(b) Morgenroth, J.: Berlin. klin. Wochschr. **54**, 55 (1917).

(85) Morgenroth, J., and Tugendreich, J.: Berlin. klin. Wochschr. **53**, 794 (1916); Biochem. Z. **79**, 257 (1917).

(86) Morgenroth, J., and Schnitzer, R.: Z. Hyg. Infektionskrankh. **103**, 441 (1924); reference 50, p. 1019.

(87) Moore, H. F., and Chesney, A. M.: Arch. Internal Med. **19**, 611 (1917); **21**, 659 (1918).

(88) Northey, E. H.: Chem. Rev. **27**, 85 (1940).

(89) Okamoto, H.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5**, 36 (1931).

(90) Okamoto, H., and Sogen, Y.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5**, 4 (1930).

(91) Oesterlin, M.: Klin. Wochschr. **15**, 957 (1936); Squibb Abstract Bull. **9**, 1855 (1936).

(92) Oesterlin, M.: Z. Hyg. Infektionskrankh. **118**, 263 (1936); Chem. Abstracts **30**, 7209 (1936).

(93) Ostromislensky, I. I.: *The Scientific Basis of Chemotherapy.* Inter-American Medical Publishing Company, New York (1926).

(94) Patterson, A. M.: J. Am. Chem. Soc. **47**, 543 (1925).

(95) Prelog, V.: Ann. **532**, 69 (1937).

(96) Prelog, V., Seiwerth, R., Heimbach-Juhasz, S., and Stern, P.: Ber. **74**, 647 (1941).

(97) Rabe, P.: Ber. **55**, 522 (1922); Ann. **492**, 242 (1932).

(98) Rabe, P., and Hagen, G.: Ber. **74**, 636 (1941).

(99) Renfrew, A. G., and Butler, C. L.: J. Am. Chem. Soc. **62**, 3304 (1940).

(100) Renfrew, A. G., and Cretcher, L. H.: J. Am. Chem. Soc. **57**, 738 (1935).

(101) REYMAN, J., AND SUSZKO, J.: Bull. Acad. Polonaise **1935A**, 360.
(102) ROSENMUND, K. W., AND KITTLER, C.: Arch. Pharm. **262**, 18 (1924).
(103) SANDERS, J. P.: J. Am. Med. Assoc. **97**, 850 (1931).
SANDERS, J. P., AND DAWSON, W. T.: J. Am. Med. Assoc. **99**, 1773 (1932).
(104) SCHMIDT, L. H., AND SESLER, C. L.: J. Pharmacol. **72**, 311 (1941).
(105) SHAFFER, P. A.: Science **89**, 547 (1939).
(106) SLOTTA, K. H., AND BEHNISCH, R.: Ber. **68**, 754 (1935).
(107) SOLIS-COHEN, S., AND GITHENS, T. S.: *Pharmacotherapeutics*. D. Appleton, New York (1928).
(108) SOLOMON, W.: (a) J. Chem. Soc. **1938**, 6; (b) J. Chem. Soc. **1941**, **77**.
(109) STUART, E. H., POWELL, H. M., ROSE, C. L., AND BIBBINS, F. E.: J. Am. Pharm. Assoc. **28**, 90 (1939).
(110) SUSZKO, J., *et al.*: Chem. Abstracts **24**, (1930) and ff.
(111) VON OETTINGEN, W. F.: *The Therapeutic Agents of the Quinoline Group*. The Chemical Catalog Company, New York (1933).
(112) WHITE, B.: *Biology of the Pneumococcus*. The Commonwealth Fund, New York (1938).
(113) WHITE, H. J., BRATTON, A. C., LITCHFIELD, J. T., AND MARSHALL, E. K.: J. Pharmacol. **72**, 112 (1941).
(114) WORK, T. S.: J. Chem. Soc. **1940**, 1315.
(115) ZETZSCHE, F., AND FREDRICH, A.: Ber. **73**, 1420 (1940).

INDOLE

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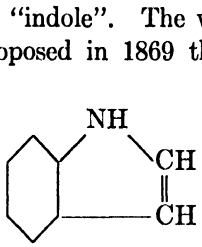
I. INTRODUCTION

In 1866, Baeyer and Knop (26), in the course of a study of the structure of indigo, reduced isatin and obtained two products, $C_8H_7NO_2$ and C_8HN_7O (dioxindole and oxindole), which they regarded as hydroxy derivatives of C_8H_7N; they named the latter "indole". The work was continued by Baeyer and Emmerling (24), who proposed in 1869 the formula which is generally accepted:



Indole

This structure was suggested largely as a result of synthetic methods of preparation of the compound by fusion of a mixture of nitrocinnamic acid, iron filings, and sodium hydroxide, and by the action of lead peroxide upon azocinnamic acid.

A system of nomenclature was devised by Baeyer (20), and used by Fischer (121), in order to differentiate the derivatives of indole. This system was cumbersome, involving independent numerical designations for each ring. Current practice is to use numbers, with the nitrogen atom as 1, and with clockwise assignment of numbers to the positions in the formula shown above. The 2- and 3-positions are occasionally referred to as the α- and β-positions, respectively.

Indole derivatives occur widespread in many natural products. Indole itself has been obtained, usually in small amounts, by extraction from naturally occurring materials by methods which suggest that the indole so obtained is in many cases the result of breakdown of its derivatives. Various plants have yielded indole, among them the following: *Robinia pseudacacia* (107), the jasmines (68, 170, 363), certain citrus plants (344), and orange blossoms (171). The usual procedure is to extract the blossoms repeatedly with a suitable solvent, followed by distillation of the extract. In this way, Hesse and Zeitschel (171) obtained about 0.1 per cent of indole from orange-blossom oil, while Hesse (170) isolated 2.5 per cent from jasmine. Herter (167) also found indole in the wood of *Celtis reticulosa.*

Indole is also found after putrefactive processes have taken place. It is found in the animal body wherever pus formation occurs (321) and in the liver and pancreas (278), the brain (371), and bile (111). It is formed in the putrefaction of milk (408), of blood fibrin (284, 347, 394), of albumin (36), and possibly of vegetable protein (170). Formation of indole from albumin may be stopped by the addition of lactose, while other sugars have varying effects upon its production (173, 355). The formation of indole is presumably the result of the decomposition of tryptophan in these cases of putrefaction of protein material.

Indole has also been found to be present in coal tar in the fraction boiling between 240° and 260°C. (396), from which it may be isolated as its solid sodium or potassium derivative after treatment with sodium amide, sodium, or potassium hydroxide. Homologous indole derivatives can also be obtained from coal tar (240). Molasses tar has also yielded some of the base (50).

II. SYNTHESES OF INDOLE

Indole was first prepared synthetically by Baeyer (15); he oxidized indigo to obtain isatin, reduced the isatin to dioxindole and oxindole using zinc dust, and further reduced oxindole to indole by passing its vapors over hot zinc oxide. Also, Baeyer (17) reduced 2,3-dichloroindole to indole.

Indoxyl, reduced by sodium amalgam, by zinc dust and alkali (387), or catalytically (187), yields indole. Dihydroindole can be obtained by the electrolytic reduction of the sulfur analog of oxindole; this in turn yields indole upon being dehydrogenated (376).

In the preparation of synthetic indigo by the method involving the fusion of phenylglycine or its *o*-carboxylic acid with sodium hydroxide, if the melt is "over-melted" a small amount of indole is obtained (89). This melt also yields indole when a metal such as sodium or iron is added at 300°C. (90). By adding sodium amalgam or zinc dust to the alkaline melt of indoxylic acid at 60–70°C. a fairly good yield of indole was obtained by Vorländer and Apelt (387).

More generally applicable methods of synthesis of indole and its derivatives involve ring closures to form the pyrrole side ring. Among these various methods, the Fischer procedure (see section IV B) is the most versatile for the preparation of derivatives. This method, discovered by E. Fischer and Jourdan (126) in 1883, involves in general the loss of ammonia from phenylhydrazones. Thus, the phenylhydrazone of pyruvic acid yields indole-2-carboxylic acid (91) and this upon decarboxylation gives indole (*vide infra*). It would be expected that acetaldehyde phenylhydrazone would form indole; this is not realized.

Other syntheses, which like the Fischer synthesis involve ring closure by the reaction of a carbon group with the ortho position, follow. Berlinerblau (42, 43, 283) employed aniline and α,β-dichloroethyl ether according to the following scheme:

$$(ClCH_2CHClOC_2H_5 \rightarrow ClCH_2CHO)$$

$$2C_6H_5NH_2 + ClCH_2CHO \rightarrow C_6H_5NHCH{=}CHNHC_6H_5 \rightarrow \text{indole}$$

Also, Prud'homme (323) used a somewhat analogous procedure with aniline and ethylene dibromide as the initial reactants:

$$C_6H_5NHCH_2CH_2NHC_6H_5 \xrightarrow{CrO_3} C_6H_5NHCH{=}CHNHC_6H_5 \xrightarrow{Zn} \text{indole}$$

Polikier (317) heated the dianilide of tartaric acid with zinc chloride to obtain indole; dianilinosuccinanilide was an intermediate. The calcium salt of phenylglycine when heated with excess of calcium formate gave indole in very small yield (264); the product was isolated as the picrate. Distilling *o*-chloro-ω-chloroacetanilide with zinc dust yielded small amounts of indole (352). Baeyer found that indole was formed when vapors of ethylaniline were led through hot tubes (22).

A rather large number of methods have been described in which ring closure is accomplished by reaction of a carbon group, attached to the benzene ring, with a nitro group, an amino group, or a substituted amino group at the ortho position. Gluud (144, 146) synthesized *o*-aldehydophenylglycine and effected ring closure (and decarboxylation of the intermediate indole-2-carboxylic acid) by heating the compound in acetic anhydride containing sodium acetate. A similar procedure (145) also gave indole. Verley (385) treated *N*-aldehydo-*o*-toluidine with sodium amide and obtained indole. The distillation of oxal-*o*-toluic acid with zinc dust, or dry distillation of the barium salt, gives indole (263). Also, *N*-methyl-*o*-toluidine, dropped on reduced nickel at 300–330°C., produces small yields of indole (65, 66, 67); other alkyltoluidines (23) also yield indoles.

A series of methods involving substituted cinnamic acids, styrenes, stilbenes, and analogous compounds as starting materials are described in the literature; examples follow. Lipp's indole synthesis involves the heating of *o*-amino-ω-chlorostyrene with sodium ethylate; reduction of the nitro group and ring closure result (249). Weermann (392, 393; also 93) has prepared indole by the use of *o*-nitrocinnamide as follows:

$$o\text{-}NO_2C_6H_4CH{=}CHCONH_2 \xrightarrow[CH_3OH]{KOCl}$$

$$o\text{-}NO_2C_6H_4CH{=}CHNHCOOCH_3 \xrightarrow[CH_3COOH]{Fe}$$

$$o\text{-}NH_2C_6H_4CH{=}CHNHCOOCH_3 \xrightarrow{KOH} \text{indole}$$

A quantitative yield of indole is reported when *o*,*o*′-diaminostilbene hydrochloride is heated under reduced pressure (94, 379). Baeyer and Emmerling (24) and Beilstein and Kuhlberg (39) prepared indole from *o*-nitrocinnamic acid. Reduction of *o*-nitrophenylacetaldehyde yields indole (392). Also, *o*-nitrophenylpyruvic acid has been converted into indole-2-carboxylic acid by Reissert (332), Kermack, Perkin, and Robinson (222), and Polyakov (320). Pschorr and Hoppe (324) prepared 2-aminoindole from *o*-nitrophenylacetic acid; this was converted into indole by reduction with sodium and alcohol. Indole may also be obtained from *o*,ω-dinitrostyrene (266, 384). Walter (390) prepared it by treating acetylated *o*-aminocinnamic acid with fuming hydrochloric acid at high temperatures. Some indole is obtained from *o*-aminophenylpropiolic acid (336).

A variety of miscellaneous methods have also been reported. In some of these cases rather strenuous treatment is involved, and for many the yields are not good. In summary, however, the following examples are cited: Morgan (274) provided one of the earliest syntheses of indole by his fusion of carbostyril with alkali. Hofmann and Königs (175) obtained indole and quinoline as the products resulting from the high-temperature treatment of tetrahydroquinoline or methyltetrahydroquinoline. Fusion of albumin with potassium hydroxide yields indole, skatole, and other products (108, 243, 280); similarly strychnine yields indole (151, 250). Also, indole-2-carboxylic acid (75), and quinoline-2,3-dicarboxylic acid (153), when heated with calcium carbonate give indole. Ketodihydrobenzene-*p*-thiazine (383) with a red-hot mixture of zinc and copper powders yields some indole. Acetylene and aniline (257) passed over metallic or oxide catalysts form indole.

Also, Fileti (120) obtained indole by passing the vapor of cumidine over hot lead oxide, and by distilling skatole over hot porcelain chips. Widmann (399) distilled 3-nitro-4-isopropenylbenzoic acid with lime and obtained a small yield of indole.

Tripyrroline (85) and pyrrole (84) have been converted into indole.

III. PROPERTIES OF INDOLE

A. Miscellaneous

Indole is a colorless crystalline solid in the form of shining leaflets. It melts at 52°C. and boils at 253°C. (corr.) at 762 mm. It is volatile with steam. It is soluble in hot water and in hot alcohol, and is also soluble in ether and in benzene. The compound in the impure state has an unpleasant odor.

Indole is very feebly basic. Generally it tends to form resin-like materials when heated with acid; heating the hydrochloric acid solution of indole yields an orange resin, which upon precipitation with water from its alcohol solution is converted to a flocculent white precipitate (75).

Mild oxidation of indole leads in most instances to the formation of indigo. Thus, indigo is formed from indole by the action of air in the presence of sulfite or bisulfite (95, 279), by monopersulfuric acid (Caro's acid) (96), by hydrogen peroxide (322), and by ozone (279). On the other hand, chromic acid causes the precipitation of a dark violet-brown solid, which is dissolved by concentrated hydrochloric acid with a color change to green (109). Also, a gray-green powder is obtained as a result of the action of ferric chloride upon indole (244).

Indole yields 3-nitrosoindole through the action of sodium nitrite and acetic acid (279) or of amyl nitrite and sodium ethylate (5, 414, 418). Through migration of the hydrogen atom from position 1, this product exists largely as the isomeric 3-oxime.

Treatment of indole with acetic anhydride yields 1-acetyl- and 1,3-diacetylindoles (415). Chloroform and sodium ethylate, or alkali, produce a mixture of indole-3-aldehyde and 3-chloroquinoline (101).

Indole reacts to form a sodium derivative with metallic sodium or sodium amide and a potassium derivative with potassium hydroxide (396). This reaction is used in separating indole from the other constituents found in the portion of coal tar boiling at 240–260°C. (141).

Also, indole will condense with aldehydes in the molecular ratio of 2 to 1 (179) and similarly with acetone (350).

B. Reduction

Indole is readily converted to 2,3-dihydroindole by electrolytic reduction or by catalytic hydrogenation. Carrasco (64) and v. Braun and Sobecki (55) have prepared the dihydroindole electrolytically. Catalytic reduction methods reported have made use most commonly of platinum, nickel, nickel salts, copper, or copper salts.

Catalytic hydrogenation under pressure produces first the dihydroindole, but absorption of hydrogen may continue, to form either octahydroindole or an aniline derivative if the pyrrole ring is fractured. The latter effect predominates if nickel is the catalyst. Thus, Willstätter, Seitz, and v. Braun (406) found that with a nickel catalyst at 225°C. no octahydroindole was formed, but that continued hydrogenation past the dihydro stage yielded 1-ethyl-2-aminocyclohexane, which v. Braun, Bayer, and Blessing (54) had erroneously thought to be octahydroindole. The true octahydroindole was obtained by Willstätter and Jaquet (405), who used platinum as the catalyst. The use of copper catalysts gives good yields of dihydroindole (188).

Treatment of molten indole at 200°C. with hydrogen in the presence of reduced nickel gives *o*-toluidine (65, 66).

C. Nitration

The simple nitro derivatives of indole are not obtained through direct nitration but are prepared by indirect methods such as those of Fischer (see section IV B) by the use of the Japp and Klingemann reaction (210, 211, 259). Oxidation of the nitrosoindoles will yield the corresponding nitro derivatives (4, 6). 3-Nitroindole, which cannot be obtained from indole by the use of nitric acid, may be prepared by the action of ethyl nitrate and sodium ethylate upon indole (6).

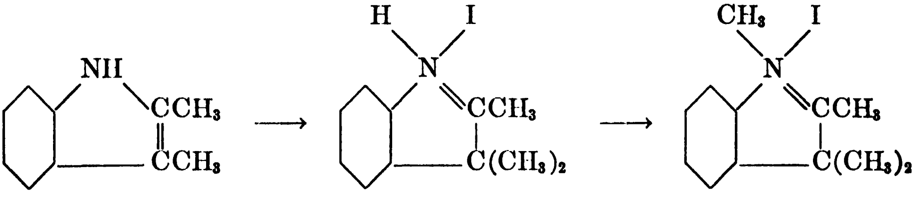
Nencki (279) treated a saturated aqueous solution of indole with fuming nitric acid and obtained a dark red solid which he designated as a "nitrosoindole nitrate" $C_{16}H_{13}(NO)N_2 \cdot HNO_3$. This product does not react like a true nitroso compound, nor does it give a positive Liebermann reaction (417).

D. Halogenation

Direct chlorination and bromination to yield indoles substituted in the benzene nucleus is not feasible; indirect methods of synthesis are necessary, as in the case of the nitro derivatives. Sulfuryl chloride, however, reacts to form 2-chloroindole (265). The addition of iodine in potassium iodide to the aqueous or alcoholic solution of indole causes the precipitation of 3-iodoindole (293, 295); in the presence of sodium bicarbonate this method yields indigo (295) also. Addition of bromine water to indole causes merely the appearance of a noncrystalline turbidity (35).

E. Alkylation

The alkylation of indole has been studied very thoroughly (71), the earliest investigations being those of E. Fischer (127, 130, 131). The difficulties encountered at first in ascribing structural formulas to the products were solved by the work of Brunner (56, 57, 58, 59) and of Plancher (73, 307, 311, 312, 313). It was assumed at first that methylation yielded a dihydroquinoline, but this conclusion was abandoned when it was shown that the 2,3-dimethylindole is first formed and that shifting of the hydrogen atom from the 1- to the 3-position allows further alkylation:



F. Chemical properties aiding in detection and identification

The pine-splinter test of Baeyer (16) consists in dipping a pine shaving into an alcoholic solution of indole which has been saturated with hydrogen chloride; the wood takes on a cherry-red coloration which changes to a brownish red. This effect is not given by indolecarboxylic acids or by 2- and 3-alkyl-substituted indoles (121). Ehrlich's sensitive test (98), which is suitable for the detection of indole in bacterial cultures and in plants, involves the addition of the solution being tested to a 2 per cent solution of *p*-dimethylaminobenzaldehyde; hydrochloric acid is added dropwise. The presence of indole is shown by the appearance of a red coloration. Herter and Foster (168) have devised another color test: a 2 per cent solution of sodium β-naphthoquinonesulfonate is added to an alkaline solution of indole, whereupon a blue-green coloration results; concentration of the solution yields blue crystals. A quantitative determination of indole by color comparison with standard solutions is based upon this method. Further, a sodium nitroprusside test (345, 353) involves adding that reagent to an indole solution; addition of alkali then causes the appearance of a deep violet-blue, which in turn upon acidification produces a pure blue.

An indole-containing solution when treated with concentrated sulfuric acid and formaldehyde gives a violet-red coloration (233). Other aldehydes may be substituted for formaldehyde in this test with the production of various colors (see table 1). Other aliphatic aldehydes also may be used (156).

Oxalic acid melted with indole produces a crimson material which remains stable in aqueous solution and is changed only slightly by alkali (147). Sugars (136, 391) give color effects upon treatment with hydrochloric acid in the presence of indole. Many other color reactions have also been found to indicate the presence of indole (see, for example, references 75, 76, 149, 273).

TABLE 1

Colors produced by the reaction of indole with aldehydes

ALDEHYDE	COLOR PRODUCED	REFERENCES
Glyoxylic acid	Red	(81, 135)
Vanillin	Orange	(277, 370)
Furfural	Orange	(135)
p-Nitrobenzaldehyde	Red	(48)
Protocatechuic aldehyde	Orange-red	(48)
Piperonal	Orange-red	(48)
Safrole	Greenish yellow	(48)
Cinnamaldehyde	Red	(48)

The formation of a picrate is suitable for the identification of indole (170); the picrate was first described by Baeyer (18, 23) as consisting of long red glistening needles soluble to an extent in benzene. Compounds are formed by indole with *sym*-trinitrobenzene (341), with sodium bisulfite (170), and with sarcosine anhydride (298).

Pauly and Gundermann (295) have found that a determination of indole by iodimetry appears to be feasible for skatole-free solutions of indole.

IV. SIMPLE DERIVATIVES OF INDOLE

A. General description

All indole derivatives show certain family resemblances to indole (121), but striking changes can be brought about by substitution of groups in the pyrrole ring. Thus, the fecal-like odor of skatole is the most pronounced of all the methylindoles, less pronounced for the 2-methylindole and the 2,3-dimethylindole; 1-methylindole, on the other hand, resembles methylaniline in odor. Introduction of carboxyl groups or phenolic hydroxyl groups causes elimination of the odor, and the naphthindoles are also without odor.

All the common indole derivatives, like indole, form well-defined crystalline picrates, yellow to red in color. Formation of picrates is usually a suitable procedure for identification and purification.

The pine-splinter test is given in varying degrees of sharpness and with the production of various colors by most indoles, failing, however, with indolecarboxylic acids and dialkylindoles having alkyl groups substituted in both the 2- and the 3-positions. Other alkyl derivatives give a cherry-red, while arylindoles, including both the phenyl- and naphthyl-indoles give a blue-violet coloration.

Nitrous acid has varying effects upon the different derivatives. 1-Methylindole reacts in a fashion similar to indole, forming a true nitroso compound. However, 2-methyl- and 2-phenyl-indoles give complex products which show no nitroso reactions. The 3-alkyl- and the 2,3-dialkyl-substituted indoles show the anticipated nitrosoamine formation at position 1.

Direct nitration of indole derivatives is generally impracticable, but some cases of successful nitration have been reported. Thus, by treating 2,3-dimethylindole with potassium nitrate and concentrated sulfuric acid at 0°C., Bauer and Strauss (34) obtained 5-nitro-2,3-dimethylindole, which proved to be identical with the product obtained by the Fischer method from the *p*-nitrophenylhydrazone of ethyl methyl ketone. Concentrated nitric acid gives a dinitro derivative with 2-methylindole (413); identical with this dinitroderivative is the compound obtained by direct nitration of 3-nitro-2-methylindole (6). Nitration of skatole gives no definite nitro derivative (34).

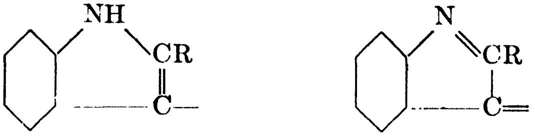
Indole-3-aldehydes can be prepared from indoles having a replaceable hydrogen at the 3-position and also with or without an alkyl or aryl group at the 2-position, by reaction with *N*-methylformanilide in the presence of condensing agents similar to phosphorus oxychloride (191). Certain indoles will also undergo the Gattermann and Hoesch aldehyde synthesis (354).

Indole-3-aldehyde was first obtained, along with harman, by Hopkins and Cole (178) as a result of treatment of tryptophan with ferric chloride. Later, Ellinger (102, 103) prepared it by treatment of indole with chloroform and potassium hydroxide; 3-chloroquinoline was also obtained in this reaction. Boyd and Robson (52) effected its preparation through the action of zinc cyanide upon 2-carbethoxyindole.

The usual condensation reactions are shown by indole-3-aldehyde. Thus, it will condense with hippuric acid to yield the azlactone which can be converted to the indoleacrylic acid (333) or to the amino acid tryptophan (104, 106). Hydantoin will also condense with the aldehyde (53, 235).

Reduction of derivatives, with the exception of the carboxylic acids, yields the dihydroindoles, and may be accomplished by electrolytic reduction, by catalytic hydrogenation, or by the use of acids and metals. The ease of reduction varies: 2,3-dimethylindole is more easily reduced than 2-methylindole, and that more easily than 1-methylindole.

Aldehydes (1 mole) will condense with 2-methylindole (2 moles), as was discovered by Fischer (123), with elimination of water to form leuco bases which yield dyes of the rosaniline type. Condensation with aldehydes may also take place in equimolecular proportions (138) to yield products in which the indole nucleus is contained in either of the following forms:



2-Methylindole has been condensed with various aldehydes (60, 123, 159, 179, 351, 386) and with ketones (350). Skatole will condense with benzaldehyde (159, 397) under rather strenuous conditions, e.g., when zinc chloride is used.

Derivatives of indole, like indole, give completely normal values for their molecular weights in benzene. In naphthalene there are anomalies, as in the cases of indole, skatole, and 2,3-dimethylindole (117).

Acids generally have less effect upon homologs of indole than upon indole itself, and fusion with potash oxidizes them to the respective carboxylic acids.

Indoles having no substituent group in the 1-position react with Grignard reagents to form the indolylmagnesium halides, in a fashion reminiscent of the pyrroles. The indolylmagnesium halides are, in turn, useful in further synthesis; with reagents common in Grignard syntheses, substitution in the 3-position results. In general, other substitution reactions commonly occur, with introduction of groups preferentially at the 3-position.

Of the simple alkyl-substituted indoles, skatole (3-methylindole) and "methylketole" (2-methylindole) deserve special mention. Of these, skatole is found in nature usually where indole is found, and is produced in bacterial cultures under conditions similar to those under which indole is formed. Methylketole, on the other hand, is essentially a synthetic product.

Skatole in the pure form is a white solid, melting at 95°C. and boiling at 266.2°C. at 760 mm.; it has a persistent fecal odor. When crystallized from water or ligroin, it is obtained as glistening leaflets. At 16°C. its solubility in water is 0.45 g. per liter.

Fusion of skatole with alkali gives indole-3-carboxylic acid (72). With sodium in a stream of carbon dioxide, skatole undergoes a Kolbe reaction with the formation of 3-methylindole-2-carboxylic acid (72). With chloroform and alcoholic alkali (103, 254, 316), or with chloroform and sodium ethylate (253, 254), 4-methyl-3-chloroquinoline is formed. Hydrochloric acid (286, 348) or sulfuric acid and acetic acid (349) will polymerize skatole to diskatole. Skatole will condense with benzaldehyde (397) and with cinnamaldehyde (87). It will form a hydrochloride (122). *sym*-Trinitrobenzene (341) and picric acid (281) will form compounds with it. Reduction of skatole with zinc dust and hydrochloric acid yields dihydroskatole (397).

Skatole may be synthesized easily by the Fischer method from the phenylhydrazone of propionaldehyde (8, 121, 122, 261) (*vide infra*); it may also be obtained from 3-nitrocumic acid (119), indigo (19), aniline and glycerol (134), and *o*-acetylanilidoacetic acid ethyl ester (63), as well as from other sources (28, 39, 218).

Methylketole (2-methylindole) is a solid, melting at 60°C. and boiling at 272°C. at 750 mm.; it has an odor like that of indole. It is soluble in alcohol, but difficultly soluble in water. It gives the pine-splinter test in the presence of hydrochloric acid. Methylketole forms 2-methyl-3-nitrosoindole with isoamyl nitrite (367); this substance can be oxidized to the corresponding nitro compound (4). It gives colored solutions with aldehydes in the presence of hydrochloric acid (156). With chloroform and sodium ethylate, 2-methyl-3-chloroquinoline is obtained, while with bromoform the corresponding bromo compound is formed (254, 255). Reduction with tin and hydrochloric acid converts methylketole to 2-methyldihydroindole (397). Chloroplatinic acid and also picric acid yield crystalline products (25).

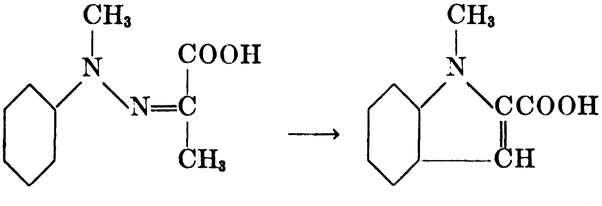
The Fischer method (*vide infra*) may be used for the preparation of 2-methylindole, employing the phenylhydrazone of acetone (121). Other methods of preparation involve aniline and monochloroacetone (283), dihydromethylketole (218), *o*-amidophenylacetone (25), and acetylated *o*-toluidine (192).

For a further summary of the properties of the simpler indole derivatives, see *Biochemisches Handlexikon*, Vol. IV, page 859.

B. The Fischer synthesis of indole derivatives

While the Fischer method may be applied somewhat indirectly to the preparation of indole (*vide supra*), it finds its chief usefulness in the preparation of many and varied indole derivatives. It is in itself very versatile, but it is made even more general by the preparation of otherwise unattainable phenylhydrazones from diazonium salts by the method of Japp and Klingemann (210, 211). In general, Fischer's synthesis may be described as involving the elimination of ammonia from phenylhydrazones, and may be accomplished with the phenylhydrazones of most carbonyl compounds the structures of which allow ring closures to occur.

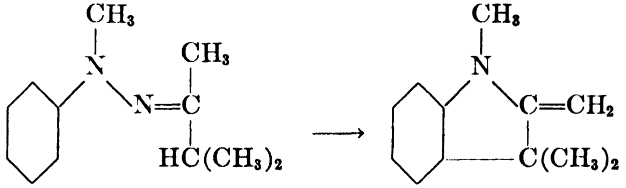
In 1883, E. Fischer and Jourdan (126) found that when they boiled the methylphenylhydrazone of pyruvic acid with alcoholic hydrogen chloride, they obtained $C_{10}H_9O_2N$ in about 5 per cent yield. Fischer and Hess (125) investigated this compound further and found it to be an indole derivative:



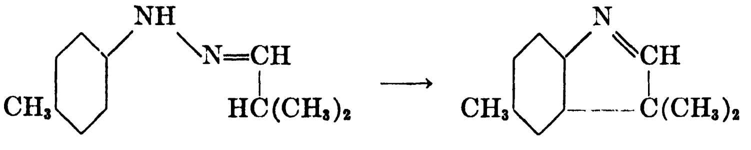
This work was continued by Fischer, who found that zinc chloride was a better catalyst for the reaction, since this catalyst allowed a wider range of applicability (122). The procedure employed by Fischer may be described by citing his preparation of 2-methylindole; this he accomplished by mixing acetone phenylhydrazone with five times its weight of anhydrous zinc chloride, and by heating the mixture to about 200°C. A yield of 60 per cent of 2-methylindole was obtained. Similarly, Fischer obtained in this way a 35 per cent yield of skatole from propionaldehyde phenylhydrazone (121).

Since the time of this early work a number of changes have been introduced into the method, with improvement in the yields. Thus it was found (91) that by the use of an inert solvent such as methylnaphthalene, and by keeping the temperature below 150°C., acetone phenylhydrazone gave a 75 per cent yield of 2-methylindole, propionaldehyde phenylhydrazone an 80 per cent yield of skatole, and the phenylhydrazone of pyruvic acid gave a 60 per cent yield of indole-2-carboxylic acid. Also, the large amounts of zinc chloride used by Fischer were shown to be unnecessary (9). The reaction was found to proceed in the presence of 1 per cent of zinc chloride; cuprous chloride, cuprous bromide, or platinum chloride may also be used. Besides these, a number of other catalysts have been employed: concentrated sulfuric acid by Nef (276), Walker (388), and Reissert and Heller (332); alcoholic sulfuric acid by Wislicenus and Arnold (409); alcoholic zinc chloride by Plancher (306). It has also been found that nickel, cobalt, and copper powder, cobalt chloride, nickel chloride and many other salts catalyze the reaction (234). Grignard reagents have also been used (154).

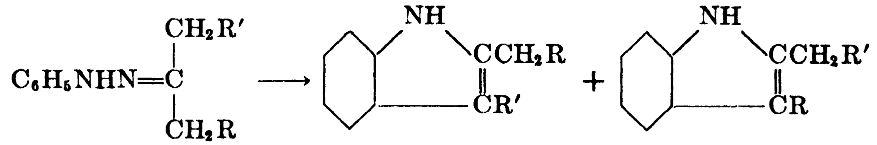
The ease of indole formation from the various phenylhydrazones varies irregularly; with some, the reaction occurs extremely readily. For example, the cyclohexanone phenylhydrazone undergoes indole formation as a result of gentle warming with aqueous hydrochloric acid. Also Plancher (306) and Jenisch (212) found that the methylphenylhydrazones of isopropyl methyl ketone and isopropyl phenyl ketone undergo ring closure even at room temperature in the presence of alcoholic zinc chloride.



Grgin (158) obtained 3,3,5-trimethylindolenine by warming the *p*-tolylhydrazone of isobutyraldehyde at 60°C. with alcoholic zinc chloride:

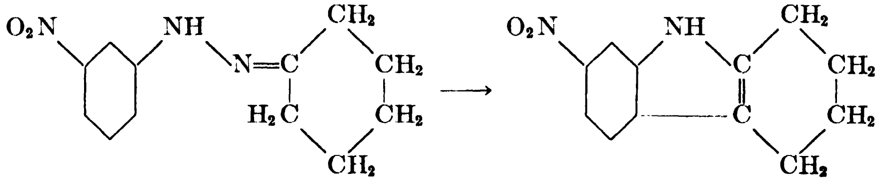


While the Fischer method is extremely varied in its applications, occasional exceptions and limitations should be noted. Acetaldehyde phenylhydrazone would be expected to yield indole, but this has not been realized. Also, phenylhydrazones of β-keto esters more commonly produce pyrazolones. The catalytic decomposition of arylhydrazones of unsymmetrical ketones would be expected in many instances to lead to a mixture of two products:



This possibility has not been investigated very extensively, but isolated reports indicate only one product in each case. For example: the phenylhydrazone of methyl propyl ketone (9, 121) gives only 2-propylindole; the phenylhydrazone of isopropyl methyl ketone gives only 2,3,3-trimethylindolenine (212, 306); when butyl methyl ketone is used, 2-methyl-3-propylindole is formed (10).

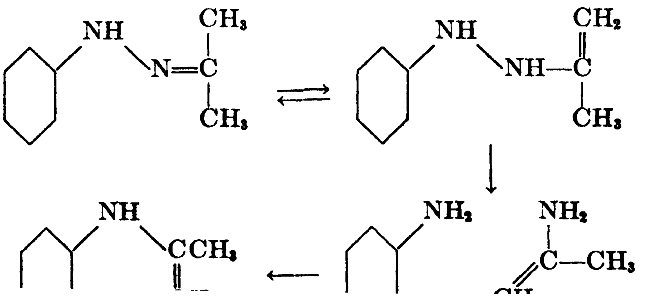
When meta-substituted phenylhydrazines are used as reagents, the possibility of two products being obtained is apparent. In some instances of such indole syntheses the mode of ring closure is not specified (92, 221, 224, 340, 381). However, Borsche found that the *m*-nitrophenylhydrazone of cyclohexanone (51) underwent ring closure to the position para to the nitro group:



The preparation of a tetrahydrocarbazole in the fashion indicated above was first accomplished by Drechsel (88), who used phenylhydrazine and cyclohexanone; Baeyer (21) identified the product as tetrahydrocarbazole. This method of synthesis has been used to quite an extent, but applies most generally to the use of the simpler cyclohexanones. A number of ketones of the terpene group will not give the reaction.

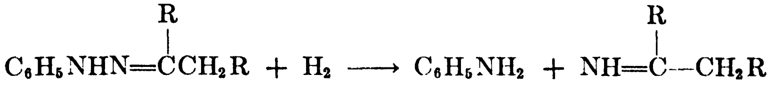
The elucidation of the mechanism of the Fischer synthesis has occupied the attention of many workers in the field. No very critical evaluation of the various postulates that have been advanced will be attempted here, but a statement of some of the suggestions will be of interest.

The mechanism proposed by R. Robinson and G. M. Robinson (337, 338) involves rearrangement before ring closure, and adapts itself quite generally to various specific cases. Its essentials follow:



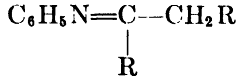
Reddelien (328), on the basis originally of his discovery of the oxidation of acetophenone phenylhydrazone by the action of phenylhydrazine to yield 2-phenylindole, proposed a mechanism which may be described as involving three separate stages:

I. Reduction of the phenylhydrazone during the simultaneous oxidation of stage III.



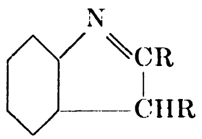
$$C_6H_5NHN{=}\overset{R}{\overset{|}{C}}CH_2R + H_2 \longrightarrow C_6H_5NH_2 + NH{=}\overset{R}{\overset{|}{C}}{-}CH_2R$$

II. Elimination of ammonia from the products of stage I to yield:



$$C_6H_5N{=}\underset{R}{\underset{|}{C}}{-}CH_2R$$

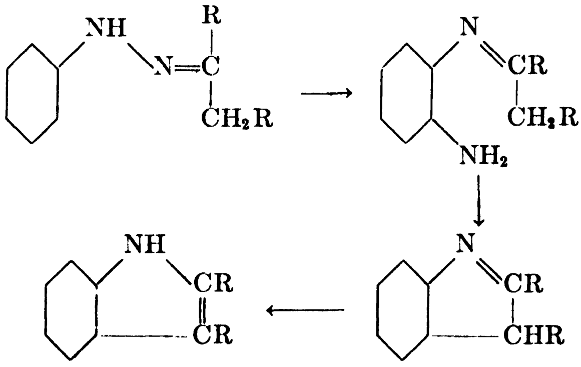
III. Oxidation of the anil of stage II by the action of the original phenylhydrazone to yield the indolenine,



which undergoes a tautomeric shift of hydrogen from position 3 to position 1, producing the indole.

This mechanism requires the assumption of an initial tautomeric shift of hydrogen in the original phenylhydrazone to yield an unsaturated substituted hydrazine, prior to stage I, if it is to explain the formation of 1-alkylindoles by the Fischer method. The Reddelien mechanism has met with considerable criticism; Robinson and Robinson (338), Bodforss (49), and Campbell and Cooper (62) have advanced substantial arguments against it.

The Cohn mechanism (77) fails to explain the fact of formation of 1-alkylindoles, and furthermore would require the formation of 6-substituted indoles from *p*-substituted phenylhydrazones:



Another mechanism, set forth by Bamberger and Landau (29), also fails to explain the formation of 1-alkyl-substituted indoles.

TABLE 2

Alkyl- and aryl-substituted indoles

INDOLES	REFERENCES*
1-Methylindole	(249, 148, 124)
2-Methylindole	(See section IV A)
3-Methylindole	(See section IV A)
5-Methylindole	(326)
6-Methylindole	(331)
7-Methylindole	(52)
1-Ethylindole	(125, 268)
2-Ethylindole	(121)
3-Ethylindole	(305, 41)
1,2-Dimethylindole	(82)
1,2-Dimethyl-5-chloroindole	(37)
1,3-Dimethylindole	(302, 82)
2,3-Dimethylindole	(121, 411, 8, 314, 219, 285)
2,5-Dimethylindole	(326, 389)
3,5-Dimethylindole	(8)
5,6-Dimethylindole	(294)
1,2,3-Trimethylindole	(82)
1,2,5-Trimethylindole	(37)
2,3,5-Trimethylindole	(412)
2,3,7-Trimethylindole	(412)
2,3,5,6-Tetramethylindole	(83)
2-Methyl-3-ethylindole	(121, 308, 285)
2-Ethyl-3-methylindole	(309)
1-Ethyl-2-methyl-5-chloroindole	(38)
1-Ethyl-2,3-dimethylindole	(412)
1-Ethyl-2,5-dimethylindole	(38)
1,3-Dimethyl-2-ethylindole	(313)
1-Propylindole	(268)
1-Isopropylindole	(268)
1-Allylindole	(270)
2-Isopropyl-3-methylindole	(310, 315)
2-*tert*-Butylindole	(313)
1-Benzylindole	(7, 86)
3-Triphenylmethylindole	(241)
1-Phenylindole	(125, 299)
2-Phenylindole	(121, 128, 304, 112, 80)
3-Phenylindole	(129)
1-Methyl-2-phenylindole	(82, 368, 80)
1-Methyl-3-phenylindole	(193)
2-Methyl-3-phenylindole	(382)
5-Methyl-2-phenylindole	(46)
7-Methyl-2-phenylindole	(46)
1,2-Diphenylindole	(299)
2,3-Diphenylindole	(121)
1,2,3-Triphenylindole	(335)
1-Methyl-2,3-diphenylindole	(47)
7-Methyl-2,3-diphenylindole	(47)
2-Anisyl-3-methylindole	(165)
2-Anisyl-3,5-dimethylindole	(165)
2-Anisyl-3,7-dimethylindole	(165)

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*Where more than one reference appears, methods of synthesis are indicated first.

TABLE 3

Acid derivatives of indole

ACID DERIVATIVES	REFERENCES*
Indole-2-carboxylic acid	(330, 74, 251, 121, 126, 303, 155, 262)
1-Methyl-2-indolecarboxylic acid	(125, 40)
3-Methyl-2-indolecarboxylic acid	(410, 110)
3-Hydroxymethyl-2-indolecarboxylic acid (lactone)	(163)
5-Methyl-2-indolecarboxylic acid	(326)
6-Methyl-2-indolecarboxylic acid	(330)
7-Methyl-2-indolecarboxylic acid	(326, 52)
5,6-Dimethyl-2-indolecarboxylic acid	(294)
1-Ethyl-2-indolecarboxylic acid	(125, 269)
1-Propyl-2-indolecarboxylic acid	(268)
1-Allyl-2-indolecarboxylic acid	(270)
1-Isopropyl-2-indolecarboxylic acid	(268)
1-Isobutyl-2-indolecarboxylic acid	(268)
1-Isoamyl-2-indolecarboxylic acid	(268)
1-Phenyl-2-indolecarboxylic acid	(125)
1-Benzyl-2-indolecarboxylic acid	(7)
Indole-3-carboxylic acid	(102, 416, 395)
1-Methyl-2-amino-3-indolecarboxylic acid	(329)
2-Methyl-3-indolecarboxylic acid	(72, 389)
1,2-Dimethyl-3-indolecarboxylic acid	(82, 226)
2,5-Dimethyl-3-indolecarboxylic acid (ethyl ester)	(389)
2,7-Dimethyl-3-indolecarboxylic acid (ethyl ester)	(389)
1-Allyl-2-methyl-3-indolecarboxylic acid	(270)
2,4-Indoledicarboxylic acid	(340)
1-Methyl-2,3-indoledicarboxylic acid	(329)
1-Benzyl-2,3-indoledicarboxylic acid	(86)
Indole-3-acetic acid	(See section V)
Indole-3-aminoacetic acid	(27)
1-Methylindole-3-acetic acid	(302, 226)
2-Methylindole-3-acetic acid	(121)
1,2-Dimethylindole-3-acetic acid	(282)
β-(Indole-1)propionic acid	(190)
β-(2-Methylindole-1)propionic acid	(190)
α-(Indole-2)propionic acid (ethyl ester)	(389)
α-(Indole-3)propionic acid	(100)
β-(Indole-3)propionic acid	(100, 190, 217, 260, 174, 419, 262)
β-(Indole-3)-α-aminopropionic acid	(See section V)
γ-(Indole-3)butyric acid	(194, 275, 419)
δ-(Indole-3)valeric acid	(258)
Indole-3-glyoxylic acid	(27)
1-Methyl-2-(3′,4′-dimethoxyphenyl)indole-3-acetic acid	(69)

*Where more than one reference appears, methods of synthesis are indicated first.

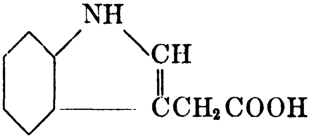
C. Alkyl, aryl, and acid derivatives of indole

The alkyl and aryl derivatives of indole are listed in table 2, while the acid derivatives are given in table 3.

V. NATURAL PRODUCTS CONTAINING THE INDOLE NUCLEUS

The indole nucleus is found to be present in a varied group of products of natural occurrence in both the animal and the vegetable kingdoms. Most of these products show marked physiological activity, and some are extremely complex in structure.

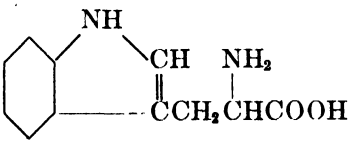
A. Heteroauxin (indole-3-acetic acid)



This acid is important in plant physiology because of the part it plays as a promoter of the growth of seedlings. It has been isolated from urine by Kögl and his collaborators (230, 231, 232); other sources of the acid are yeast and moulds. The compound is soluble in alcohol, ether, benzene, and slightly soluble in cold water. Crystallized from water or benzene it is obtained as leaflets, melting at 165°C. When heated above its melting point, the compound loses carbon dioxide to form skatole. As a color test, the Adamkiewicz-Hopkins reaction can be used (407).

Heteroauxin can be obtained by bacterial action upon tryptophan (166), albumin (346), or milk (169). A convenient synthesis, however, is by the method of Majima and Hoshino (256), who treated indolylmagnesium iodide with chloroacetonitrile and hydrolyzed the resulting indole-3-acetonitrile. Another method of synthesis is that of King and L'Ecuyer (226), who used as starting materials benzenediazonium chloride and α-acetoglutaric acid ethyl ester; the successive steps of hydrolysis, Fischer indole formation, and partial decarboxylation produce the product. The acid has also been synthesized by Tanaka (377, 378). Indole-3-acetic acids, in general, may be prepared from indoles having an unsubstituted 3-position through the reaction of formaldehyde and hydrocyanic acid and subsequent hydrolysis of the resulting nitrile (189).

B. Tryptophan



Tryptophan, one of the essential amino acids, as isolated from natural sources exists as the levo isomer in the form of colorless hexagonal leaflets. Various melting points have been reported for the levo form (273°C., 289°C., 293°C.), while for the racemic mixture melting points about 30° lower have been noted. Dry distillation of tryptophan results in decomposition, with the formation of both indole and skatole, although strong heating yields indole alone.

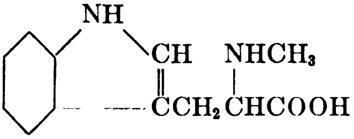
Oxidation with ferric chloride gives two products, C_9H_7NO (indole-3-aldehyde) and $C_{12}H_{10}N_2$ (harman), while ozone and chromic acid cause breakdown of the molecule. Ultraviolet light causes the formation of indole-3-acetic acid (45).

Tryptophan gives several well-defined color reactions. Thus, chlorine or bromine water produces a red-violet coloration (44, 380), while the Adamkiewicz reaction (1, 2), Ehrlich's reaction (99), and the pine-splinter test also apply.

Tryptophan as the levo isomer may be isolated from certain proteins, but such hydrolytic procedure requires care in the choice of agents. For example, acid agents yield condensation products (60), and barium hydroxide gives the racemic mixture. Enzymatic hydrolysis using trypsin (288) yields *l*-tryptophan, and it can also be obtained from casein (78). In both of these latter cases use is made of the formation of an insoluble compound with mercuric sulfate. There are many sources from which tryptophan may be obtained (177, 247, 248, 290, 291, 292, 334).

The first synthesis of *dl*-tryptophan was accomplished by Ellinger and Flamand (104, 106), who carried out an Erlenmeyer synthesis on indole-3-aldehyde. This was condensed with hippuric acid in the presence of acetic anhydride and sodium acetate to yield the azlactone, which upon subsequent hydrolysis and reduction gave the racemic mixture. A similar synthesis by Kotake (235) employed indole-3-aldehyde and hydantoin; this method was improved by modifications introduced by Boyd and Robson (53).

C. "Abrine"[1] *(d-α-methylamino-β-(indolyl-3)propionic acid)*



This compound was first encountered as a result of its isolation from the seeds of *Abrus precatorius* Linn (398). Later, in 1932, it was isolated in pure state from the same source and investigated by Ghatak and Kaul (142, 143). Its structure was assigned by Hoshino (180) after he had accomplished decarboxylation of the compound and identified the resultant as monomethyltryptamine, which he had previously synthesized (182, 183). "Abrine" and tryptophan upon complete methylation both yield the same product (343).

"Abrine" in the *d*-form after crystallization from water as colorless prisms melts with decomposition at 295°C.; the *dl*-form melts at 245°C. with decomposition (271).

The compound has been synthesized by Gordon and Jackson (152) and by Miller and Robson (271), starting with indole-3-aldehyde and 1-methylhydantoin. These compounds condensed under the influence of piperidine to form

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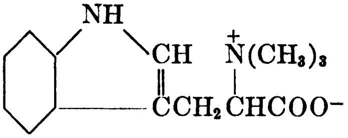
[1] It has been suggested (271) that the name abrine be dropped from use to avoid confusion with "abrin," which has been applied for years to a mixture of two poisonous proteins, a paraglobulin and a phytalbuminose, obtained from *Abrus precatorius* Linn.

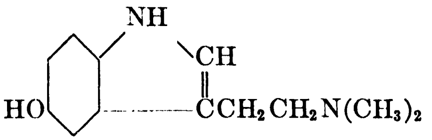
5-(indolal-3′)-1-methylhydantoin. Reduction to the indolyl product and subsequent hydrolysis completed the synthesis of the *dl*-compound.

D. Hypaphorine



Hypaphorine was discovered by Greshoff (157) in the seeds of *Erythrina hypaphorus* Boerl. The name and molecular formula ($C_{14}H_{18}O_2N_2$) were assigned by Greshoff. The study of its constitution was undertaken by van Romburgh (343) who obtained, by the methylation of tryptophan, a compound resembling hypaphorine closely; later van Romburgh and Barger obtained complete proof of its identity (343). The racemic form has been synthesized by Cahill and Jackson (61).

E. Bufotenine

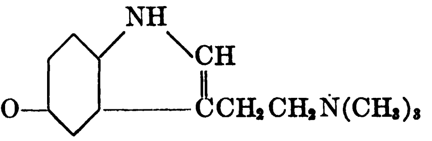


Phisalix and Bertrand in 1893 (300, 301) obtained an amorphous base from the secretions of *Bufo vulgaris* which they named bufotenine. Later, in 1920, Handovsky (162) obtained the base from the same source as a crystalline compound. Later methods of isolation made use of the formation of an insoluble flavianate (213).

The constitution of bufotenine was investigated and established by Wieland and coworkers (400, 402) through the synthesis, from 5-methoxyindolyl-3-acetonitrile, of the product of methylation of bufotenine. Hoshino and Shimodaira (183, 184) have synthesized bufotenine itself from the same acetonitrile derivative.

Bufotenine crystallizes from acetone–ether solutions; it melts at 146–147°C., and may be distilled without decomposition at 320°C. at 0.1 mm. Its picrate melts at 178°C. (402). Bufotenine has a pronounced effect upon the blood pressure (70).

F. Bufotenidine



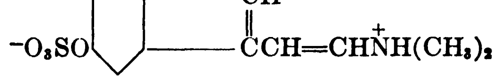
Bufotenidine was isolated as the flavianate in 1931 by Mittasch (400) from the extract of the Chinese toad. Its structural relationship to bufotenine was shown by Wieland, Konz, and Mittasch (402); methylation of both compounds yielded the same product.

TABLE 4

Some complex natural products containing the indole or dihydroindole grouping

PRODUCTS	REFERENCES
Harmine	(259, 366, 3, 164, 296, 133, 132, 139)
Harman	(133, 178, 220, 259, 204, 366, 3, 364, 365, 296, 297)
Harmaline	(259, 366, 132, 223, 140, 133)
Harmalol	(150, 133)
Harmol	(133, 325)
Harmalan (dihydroharman)	(259, 366)
Norharman	(220)
Physostigmine (eserine)	(216, 215, 319, 185, 369, 172)
Eseroline	(216, 319)
Geneserine	(318)
Eserethole	(182, 215, 216, 227, 181, 369)
Desoxyeseroline	(215)
Noreserethole	(339)
Noresermethole	(228)
Methyleserethole	(229)
Ergotoxine	(30, 31, 357, 197, 198, 202, 79)
Ergotinine	(30, 31, 357, 197, 198, 79)
Ergotamine	(357, 198, 202, 79)
Ergotaminine	(357, 198, 79)
Ergonovine	(97, 225, 372, 373, 199, 374, 79, 375, 356)
Ergometrinine	(361, 79, 374)
Ergine	(358, 359, 196, 360, 79)
Isoergine	(362, 79)
Lysergic acid	(195, 196, 360, 200, 225, 201, 203, 206, 362, 208, 207, 209, 205)
Isolysergic acid	(362, 79)
Yohimbine	(161, 137, 32, 118, 327, 33, 160)
Evodiamine	(13, 14, 12)
Rutecarpine	(11, 12, 287)
Strychnine	(267, 246, 237, 238, 239, 236, 272, 176, 245)
Brucine	(267, 246, 176, 245)

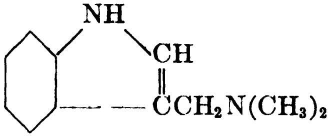
G. Bufothionine



In 1930, Wieland and Vocke (403) isolated a compound, containing both nitrogen and sulfur, from the extract of skins of Japanese toads and gave the substance the name bufothionine. It has also been isolated from the skin of the South American toad, *Bufo arenarum* (402), and from *Bufo marinus* (214).

Hydrolysis of the compound produces sulfuric acid with the formation of a compound having phenolic properties. Wieland (404) showed the relationship of the latter compound (dehydrobufotenine) to bufotenine by its hydrogenation to yield bufotenine. No synthesis of bufothionine has been reported.

H. Gramine (donaxine)



Von Euler and Hellström (114) in 1932 isolated a base from the germ of Swedish barley which they recognized as an indole derivative from the spectroscopic measurements of Hellström (115). Later, in 1935, the substance was named gramine (116); in the same year Orechoff and Norkina (289) reported isolation of a similar substance from the Asiatic reed, *Arundo donax* L., and named their substance donaxine. Von Euler, Erdtman, and Hellström (113) showed the two compounds to be identical. The constitution of gramine was established, after several attempts at its degradation (252), by the synthesis of Wieland and Hsing (401), using indolyl-1-magnesium iodide. Kühn and Stein (242) prepared it by condensing indole with formaldehyde and dimethylamine.

I. More complex natural products

Among the more complex naturally occurring products of the alkaloid type, and compounds derived from them, there are many which include in their structures the indole or dihydroindole grouping. A detailed consideration of the chemistry of these compounds falls outside the scope of this review, but the names of a number of these products and references to principal articles concerning them are included in table 4.

REFERENCES

(1) Adamkiewicz, A.: Arch. ges. Physiol. (Pflügers) **9**, 157 (1874).
(2) Adamkiewicz, A.: Ber. **8**, 161 (1875).
(3) Akabori, S., and Saito, K.: Ber. **63**, 2245 (1930).
(4) Angeli, A., and Angelico, F.: Gazz. chim. ital. **30**, ii, 274 (1900).
(5) Angeli, A., and Marchetti, G.: Atti. accad. Lincei [5] **16**, i, 381 (1909).
(6) Angelico, F., and Velardi, G.: Atti. accad. Lincei [5] **13**, i, 241 (1904).
(7) Antrick, O.: Ann. **227**, 362 (1885).
(8) Arbuzov, A. E., and Tichwinsky, W. M.: Ber. **43**, 2301 (1910).
(9) Arbuzov, A. E., and Tikhvinskii, W. M.: J. Russ. Phys. Chem. Soc. **45**, 69, 694 (1915).
(10) Arbuzov, A. E., Zaitzev, I. A., and Razumov, A. I.: Ber. **68B**, 1792 (1935).
(11) Asahina, Y., Irie, T., and Ohta, T.: J. Pharm. Soc. Japan **543**, 51 (1927).
(12) Asahina, Y., and Mayeda, S.: J. Pharm. Soc. Japan **416**, 871 (1916).
(13) Asahina, Y., and Ohta, T.: Ber. **61B**, 319 (1928).
(14) Asahina, Y., and Ohta, T.: Ber. **61B**, 869 (1928).
(15) Baeyer, A.: Ber. **1**, 17 (1868).
(16) Baeyer, A.: Ann. Supp. **7**, 56 (1870).

(17) Baeyer, A.: Ber. **12,** 459 (1879).
(18) Baeyer, A.: Ber. **12,** 1314 (1879).
(19) Baeyer, A.: Ber. **13,** 2339 (1880).
(20) Baeyer, A.: Ber. **17,** 960 (1884).
(21) Baeyer, A.: Ann. **278,** 88 (1894).
(22) Baeyer, A., and Caro, H.: Ber. **10,** 692 (1877).
(23) Baeyer, A., and Caro, H.: Ber. **10,** 1262 (1877).
(24) Baeyer, A., and Emmerling, A.: Ber. **2,** 679 (1869).
(25) Baeyer, A., and Jackson, O. R.: Ber. **13,** 187 (1880).
(26) Baeyer, A., and Knop, C. A.: Ann. **140,** 1 (1866).
(27) Baker, J. W.: J. Chem. Soc. **1940,** 458.
(28) Bamberger, E., and Kitschelt, M.: Ber. **27,** 3424 (1894).
(29) Bamberger, E., and Landau, A.: Ber. **52,** 1097 (1919) (footnote).
(30) Barger, G., and Carr, F. H.: J. Chem. Soc. **91,** 337 (1907).
(31) Barger, G., and Ewins, A. J.: J. Chem. Soc. **113,** 235 (1918).
(32) Barger, G., and Field, E.: J. Chem. Soc. **107,** 1025 (1915).
(33) Barger, G., and Scholz, C.: J. Chem. Soc. **1933,** 614.
(34) Bauer, H., and Strauss, E.: Ber. **65B,** 308 (1932).
(35) Baumann, E.: Z. physiol. Chem. **1,** 62 (1877).
(36) Baumann, E.: Z. physiol. Chem. **7,** 282 (1883).
(37) Bayer, F., and Co.: German patent 128,660.
(38) Bayer, F., and Co. (Elberfeld): German patent 137,117.
(39) Beilstein, F., and Kuhlberg, A.: Ann. **163,** 141 (1872).
(40) Benedicenti, A.: Z. physiol. Chem. **53,** 183 (1907).
(41) Benedicenti, A.: Z. physiol. Chem. **53,** 190 (1907).
(42) Berlinerblau, J.: Monatsh. **8,** 180 (1887).
(43) Berlinerblau, J., and Polikier, H.: Monatsh. **8,** 187 (1887).
(44) Bernard, C.: Compt. rend. Supp. **1,** 379 (1856).
(45) Berthelot, A., and Amoureux, G.: Compt. rend. **206,** 699 (1938).
(46) Bischler, A.: Ber. **25,** 2860 (1892).
(47) Bischler, A., and Fireman, P.: Ber. **26,** 1336 (1893).
(48) Blumenthal, F.: Biochem. Z. **19,** 521 (1909).
(49) Bodforss, S.: Ber. **58B,** 775 (1925).
(50) Boes, J.: Pharm. Ztg. **47,** 131 (1902).
(51) Borsche, W.: Ann. **359,** 49 (1908).
(52) Boyd, W. J., and Robson, W.: Biochem. J. **29,** 555 (1935).
(53) Boyd, W. J., and Robson, W.: Biochem. J. **29,** 2256 (1935).
(54) Braun, J. v., Bayer, O., and Blessing, G.: Ber. **57B,** 392 (1924).
(55) Braun, J. v., and Sobecki, W.: Ber. **44,** 2158 (1911).
(56) Brunner, K.: Monatsh. **17,** 253, 479 (1896).
(57) Brunner, K.: Monatsh. **18,** 95, 527 (1897).
(58) Brunner, K.: Monatsh. **21,** 156 (1900).
(59) Brunner, K.: Monatsh. **27,** 1183 (1906).
(60) Burr, G. O., and Gortner, R. A.: J. Am. Chem. Soc. **46,** 1224 (1924).
(61) Cahill, W. M., and Jackson, R. W.: J. Biol. Chem. **126,** 627 (1938).
(62) Campbell, N., and Cooper, R. C.: J. Chem. Soc. **1935,** 1208.
(63) Camps, R.: Ber. **32,** 3234 (1899).
(64) Carrasco, O.: Gazz. chim. ital. **38,** ii, 301 (1908).
(65) Carrasco, O., and Padoa, M.: Gazz. chim. ital. **36,** ii, 512 (1906).
(66) Carrasco, O., and Padoa, M.: Atti accad. Lincei [5] **15,** i, 699 (1906).
(67) Carrasco, O., and Padoa, M.: Atti accad. Lincei [5] **15,** ii, 729 (1906).
(68) Cerighelli, R.: Compt. rend. **179,** 1193 (1924).
(69) Chalmers, A. J., and Lions, F.: J. Proc. Roy. Soc. N. S. Wales **67,** 178 (1933).

(70) Chen, K. K., Jensen, H., and Chen, A. L.: Proc. Soc. Exptl. Biol. Med. **29**, 908 (1932).
(71) Ciamician, G.: Ber. **37**, 4227 (1904).
(72) Ciamician, G., and Magnanini, G.: Ber. **21**, 671, 1925 (1888).
(73) Ciamician, G., and Plancher, G.: Ber. **29**, 2475 (1896).
(74) Ciamician, G., and Zatti, C.: Ber. **21**, 1929 (1888).
(75) Ciamician, G., and Zatti, C.: Ber. **22**, 1976 (1889).
(76) Ciusa, R.: Atti accad. Lincei [5] **18**, ii, 100 (1909).
(77) Cohn, G.: *Die Carbazolgruppe*, p. 12. Thieme, Leipzig (1919).
(78) Cox, G. J., and King, H.: *Organic Syntheses*, Vol. X, p. 100. John Wiley and Sons, Inc., New York (1930).
(79) Craig, L. C., Shedlovsky, T., Gould, R. G., Jr., and Jacobs, W. A.: J. Biol. Chem. **125**, 289 (1938).
(80) Culman, J.: Ber. **21**, 2595 (1888).
(81) Dakin, H. D.: J. Biol. Chem. **2**, 289 (1906).
(82) Degen, J.: Ann. **236**, 151 (1886).
(83) Dennstedt, M.: Ber. **22**, 1922 (1889).
(84) Dennstedt, M.: German patent 125,489 (1901).
(85) Dennstedt, M., and Voigtländer, F.: Ber. **27**, 476 (1894).
(86) Diels, O., and Reese, J.: Ann. **519**, 147 (1935).
(87) Dostal, V.: Chem. Listy **31**, 250 (1937).
(88) Drechsel, E.: J. prakt. Chem. **38**, 65 (1888).
(89) D. R. patent 260,327; English patent 14,943.
(90) D. R. patent 152,683 (Badische Anilin-u-Soda-Fabrik (1902)).
(91) D. R. patent 238,138 (1911).
(92) D. R. patent 38,784.
(93) D. R. patent 213,713 (R. A. Weerman (1908)).
(94) D. R. patent 84,578 (Farbenfabriken vorm. F. Bayer and Co. (1895)).
(95) D. R. patent 130,629 (Badische Anilin-u-Soda-Fabrik (1901)).
(96) D. R. patent 132,405 (Badische Anilin-u-Soda-Fabrik (1901)).
(97) Dudley, H. W., and Moir, J. C.: Brit. Med. J. **1935**, I, 520.
(98) Ehrlich, P.: Deut. med. Wochschr., April (1901).
(99) Ehrlich, P.: Deut. med. Wochschr., April (1901).
(100) Ellinger, A.: Ber. **38**, 2884 (1905).
(101) Ellinger, A.: Ber. **39**, 2515 (1906).
(102) Ellinger, A.: Ber. **39**, 2518 (1906).
(103) Ellinger, A., and Flamand, C.: Ber. **39**, 4388 (1906).
(104) Ellinger, A., and Flamand, C.: Ber. **40**, 3029 (1907).
(105) Ellinger, A., and Flamand, C.: Z. physiol. Chem. **54**, 15 (1908).
(106) Ellinger, A., and Flamand, C.: Z. physiol. Chem. **55**, 8 (1908).
(107) Elze, F.: Chem.-Ztg. **34**, 814 (1910).
(108) Engler, C., and Janecke: Ber. **9**, 1411 (1876).
(109) Engler, C., and Janecke: Ber. **9**, 1414 (1876).
(110) Erlenmeyer, E., Jr., and Arbenz, E.: Ann. **337**, 302 (1904).
(111) Ernst, C.: Z. physiol. Chem. **16**, 208 (1892).
(112) Étard, M. A.: Bull. soc. chim. **39**, 531 (1883).
(113) Euler, H. v., Erdtman, H., and Hellström, H.: Ber. **69**, 743 (1936).
(114) Euler, H. v., and Hellström, H.: Z. physiol. Chem. **208**, 43 (1932).
(115) Euler, H. v., and Hellström, H.: Z. physiol. Chem. **217**, 23 (1933).
(116) Euler, H. v., Hellström, H., and Löfgren, N.: Z. physiol. Chem. **234**, 151 (1935).
(117) Ferratini, A., and Garelli, F.: Atti accad. Lincei [5] **1**, ii, 54 (1892).
(118) Field, E.: J. Chem. Soc. **123**, 3003 (1923).
(119) Fileti, M.: Gazz. chim. ital. **13**, 350 (1881).
(120) Fileti, M.: Ber. **16**, 2928 (1884).

(121) Fischer, E.: Ann. **236**, 116 (1886).
(122) Fischer, E.: Ber. **19**, 1567 (1886).
(123) Fischer, E.: Ber. **19**, 2988 (1886).
(124) Fischer, E.: Ann. **242**, 382 (1887).
(125) Fischer, E., and Hess, O.: Ber. **17**, 559 (1884).
(126) Fischer, E., and Jourdan, F.: Ber. **16**, 2241 (1883).
(127) Fischer, E., and Meyer, J.: Ber. **23**, 2629 (1890).
(128) Fischer, E., and Schmidt, T.: Ber. **21**, 1071 (1888).
(129) Fischer, E., and Schmidt, T.: Ber. **21**, 2811 (1888).
(130) Fischer, E., and Stecke, A.: Ann. **242**, 348 (1887).
(131) Fischer, E., and Stecke, A.: Ber. **20**, 2199 (1887).
(132) Fischer, O.: Chem. Zentr. **1901**, I, 957.
(133) Fischer, O.: Ber. **47**, 99 (1914).
(134) Fischer, O., and German, L.: Ber. **16**, 710 (1883).
(135) Fleig, C.: Compt. rend. soc. biol. **65**, 283 (1908).
(136) Fleig, C.: J. pharm. chim. [6] **28**, 385 (1908).
(137) Fourneau, E., and Page, H. J.: Bull. sci. pharmacol. **21**, 7 (1914).
(138) Freund, M., and Lebach, G.: Ber. **38**, 2640 (1905).
(139) Fritzsche, J.: Ann. **64**, 360 (1847).
(140) Fritzsche, J.: Ann. **92**, 330 (1854).
(141) Gesellschaft für Teerverwertung (Duisburg-Niederich): German patent 223,304.
(142) Ghatak, N.: Bull. Acad. Sci. United Provinces Agra Oudh, India **3**, 295 (1934).
(143) Ghatak, N., and Kaul, R.: J. Indian Chem. Soc. **9**, 383 (1932).
(144) Gluud, W.: J. Chem. Soc. **103**, 1254 (1913).
(145) Gluud, W.: German patent 287,282 (1913).
(146) Gluud, W.: Ber. **48**, 420 (1915).
(147) Gnezda, J.: Compt. rend. **128**, 1584 (1899).
(148) Gnezda, J.: Compt. rend. **148**, 485 (1909).
(149) Gnezda, J.: Rad. **244**, 13; Chem. Zentr. **1935**, I, 3574 (1935).
(150) Goebel, F.: Ann. **38**, 363 (1841).
(151) Goldschmitt, H.: Ber. **15**, 1977 (1882).
(152) Gordon, W. G., and Jackson, R. W.: J. Biol. Chem. **110**, 151 (1935).
(153) Graebe, C., and Caro, H.: Ber. **13**, 99 (1880).
(154) Grammaticakis, P.: Compt. rend. **204**, 502 (1937).
(155) Granacher, C., Mahal, A., and Gero, M.: Helv. Chim. Acta **7**, 579 (1924).
(156) Granström, E.: Beiträge chem. Physiol. Pathol. **11**, 132 (1908).
(157) Greshoff, M.: Mededeel. Lands Plantentium **7**, 29 (1890).
(158) Grgin, D. J.: Monatsh. **27**, 731 (1906).
(159) Hadano, M.: J. Pharm. Soc. Japan **48**, 919 (1928).
(160) Hahn, G., Kopples, E., and Ludewig, H.: Ber. **67B**, 686 (1934).
(161) Hahn, G., and Werner, H.: Ann. **520**, 123 (1935).
(162) Handovsky, H.: Arch. exptl. Path. Pharmakol. **86**, 138 (1920).
(163) Harradence, R. H., and Lions, F.: J. Proc. Roy. Soc. N. S. Wales **72**, 221 (1939).
(164) Harvey, D. G., and Robson, W.: J. Chem. Soc. **1938**, 97.
(165) Hell, C., and Cohen, H.: Ber. **37**, 870 (1904).
(166) Herter, C. A.: J. Biol. Chem. **4**, 253 (1908).
(167) Herter, C. A.: J. Biol. Chem. **5**, 489 (1909).
(168) Herter, C. A., and Foster, M. L.: J. Biol. Chem. **1**, 257 (1905).
(169) Herter, C. A., and Kendall, A. J.: J. Biol. Chem. **5**, 439 (1909).
(170) Hesse, A.: Ber. **37**, 1457 (1904).
(171) Hesse, A., and Zeitschel, O.: J. prakt. Chem. [2] **66**, 481 (1902).
(172) Hesse, O.: Ann. **141**, 82 (1867).
(173) Hirschler, A.: Z. physiol. Chem. **10**, 306 (1886).

(174) Hitchcock, A. E.: Contrib. Boyce Thompson Inst. **7**, 87 (1935).
(175) Hoffmann, L., and Königs, W.: Ber. **16**, 727 (1883).
(176) Holmes, H. L., and Robinson, R.: J. Chem. Soc. **1939**, 603.
(177) Hopkins, F. G., and Cole, S. W.: J. Physiol. **27**, 418 (1902).
(178) Hopkins, F. G., and Cole, S. W.: J. Physiol. **29**, 451 (1903).
(179) Hoschek, A. B.: Ber. **49**, 2584 (1916).
(180) Hoshino, T.: Ann. **520**, 31 (1935).
(181) Hoshino, T., and Kobayashi, T.: Proc. Imp. Acad. (Tokyo) **10**, 99 (1934).
(182) Hoshino, T., and Kobayashi, T.: Ann. **520**, 11 (1935).
(183) Hoshino, T., and Shimodaira, K.: Ann. **520**, 19 (1935).
(184) Hoshino, T., and Shimodaira, K.: Bull. Chem. Soc. Japan **11**, 221 (1936).
(185) Hoshino, T., and Tamura, K.: Ann. **500**, 42 (1932).
(187) I. G. Farbenindustrie A.-G. (C. Schumann, E. Muench, and B. Christ): U. S. patent 1,891,057 (1933).
(188) I. G. Farbenindustrie, A.-G.: German patent 623,693 (1935).
(189) I. G. Farbenindustrie, A.-G.: French patent 841,676 (1939).
(190) I. G. Farbenindustrie A.-G.: French patent 48,570 (1938).
(191) I. G. Farbenindustrie A.-G.: German patent 614,325 (1935).
(192) Imperial Chemical Industries, Ltd. (H. A. Piggott, and E. H. Rodd): British patent 330,332 (1929).
(193) Ince, W. H.: Ann. **253**, 38 (1889).
(194) Jackson, R. W., and Manske, R. H. F.: J. Am. Chem. Soc. **52**, 5029 (1930).
(195) Jacobs, W. A., and Craig, L. C.: J. Biol. Chem. **104**, 547 (1934).
(196) Jacobs, W. A., and Craig, L. C.: J. Biol. Chem. **106**, 393 (1934).
(197) Jacobs, W. A., and Craig, L. C.: J. Am. Chem. Soc. **57**, 383 (1935).
(198) Jacobs, W. A., and Craig, L. C.: Science **81**, 256 (1935).
(199) Jacobs, W. A., and Craig, L. C.: Science **82**, 16 (1935).
(200) Jacobs, W. A., and Craig, L. C.: J. Am. Chem. Soc. **57**, 960 (1935).
(201) Jacobs, W. A., and Craig, L. C.: J. Biol. Chem. **111**, 455 (1935).
(202) Jacobs, W. A., and Craig, L. C.: J. Org. Chem. **1**, 245 (1936).
(203) Jacobs, W. A., and Craig, L. C.: J. Biol. Chem. **113**, 767 (1936).
(204) Jacobs, W. A., and Craig, L. C.: J. Biol. Chem. **113**, 759 (1936).
(205) Jacobs, W. A., and Craig, L. C.: J. Am. Chem. Soc. **60**, 1701 (1938).
(206) Jacobs, W. A., Craig, L. C., and Rothen, A.: Science **83**, 166 (1936).
(207) Jacobs, W. A., and Gould, R. G., Jr.: J. Biol. Chem. **120**, 141 (1937).
(208) Jacobs, W. A., and Gould, R. G., Jr.: Science **85**, 248 (1937).
(209) Jacobs, W. A., and Gould, R. G., Jr.: J. Biol. Chem. **126**, 67 (1938).
(210) Japp, F. R., and Klingemann, F.: Ber. **21**, 549 (1888).
(211) Japp, F. R., and Klingemann, F.: Ann. **247**, 218 (1888).
(212) Jenisch, G.: Monatsh. **27**, 1223 (1906).
(213) Jensen, H., and Chen, K. K.: Ber. **65**, 1310 (1932).
(214) Jensen, H., and Chen, K. K.: J. Biol. Chem. **116**, 87 (1936).
(215) Julian, P. L., and Pikl, J.: J. Am. Chem. Soc. **57**, 539 (1935).
(216) Julian, P. L., and Pikl, J.: J. Am. Chem. Soc. **57**, 755 (1935).
(217) Kalb, L., Schweizer, F., and Schimpf, G.: Ber. **59B**, 1858 (1926).
(218) Kann, M., and Tafel, J.: Ber. **27**, 826 (1894).
(219) Keimatsu, S., and Sugasawa, S.: J. Pharm. Soc. Japan **48**, 755 (1928).
(220) Kermack, W. O., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **119**, 1602 (1921).
(221) Kermack, W. O., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **119**, 1622, 1636 (1921).
(222) Kermack, W. O., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **119**, 1625 (1921).

(223) Kermack, W. O., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **121**, 1872 (1922).

(224) Kermack, W. O., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **121**, 1880 (1922).

(225) Kharasch, M. S., and Legault, R. R.: J. Am. Chem. Soc. **57**, 1140 (1935).

(226) King, F. E., and L'Ecuyer, P.: J. Chem. Soc. **1934**, 1901.

(227) King, F. E., Liquori, M., and Robinson, R.: J. Chem. Soc. **1934**, 1416.

(228) King, F. E., and Robinson, R.: J. Chem. Soc. **1932**, 1433.

(229) Kobayashi, T.: Ann. **539**, 213 (1939).

(230) Kögl, F., Haagen-Smit, A. S., and Erxleben, H.: Z. physiol. Chem. **228**, 90 (1934).

(231) Kögl, F., Haagen-Smit, A. S., and Erxleben, H.: Z. physiol. Chem. **228**, 104 (1934).

(232) Kögl, F., Haagen-Smit, A. S., and Erxleben, H.: Z. physiol. Chem. **228**, 113 (1934).

(233) Konto, K.: Z. physiol. Chem. **48**, 185 (1906).

(234) Koraczynski, A., and Kierzek, L.: Gazz. chim. ital. **55**, 361 (1925).

(235) Kotake, M.: Ber. **55B**, 3859 (1922).

(236) Kotake, M.: Proc. Imp. Acad. (Tokyo) **12**, 99 (1936).

(237) Kotake, M., and Mitsuwa, T.: Sci. Papers Inst. Phys. Chem. Research (Tokyo) **24**, 119 (1934).

(238) Kotake, M., and Mitsuwa, T.: J. Chem. Soc. Japan **57**, 228 (1936).

(239) Kotake, M., and Mitsuwa, T.: Bull. Chem. Soc. Japan **11**, 23 (1936).

(240) Kruber, O.: Ber. **59B**, 2752 (1926).

(241) Kubota, T.: J. Chem. Soc. Japan **59**, 399 (1938).

(242) Kühn, H., and Stein, O.: Ber. **70B**, 567 (1937).

(243) Kühne, W.: Ber. **8**, 206 (1875).

(244) Ladenburg, A.: Ber. **10**, 1131 (1877).

(245) Leuchs, H.: Ber. **72B**, 1588 (1939).

(246) Leuchs, H., and Overberg, H. S.: Ber. **66B**, 951 (1933).

(247) Levene, P. A., and Beatty, W. A.: Biochem. Z. **4**, 299 (1907)

(248) Levene, P. A., and Van Slyke, D. D.: Biochem. Z. **13**, 440 (1908).

(249) Lipp, A.: Ber. **17**, 2507 (1884).

(250) Loebisch, W. F., and Schoop, P.: Monatsh. **7**, 91 (1886).

(251) Madelung, W.: Ber. **45**, 3521 (1912).

(252) Madinaveitia, J.: J. Chem. Soc. **1937**, 1927.

(253) Magnanini, G.: Ber. **17**, 246 (1884).

(254) Magnanini, G.: Ber. **20**, 2608 (1887).

(255) Magnanini, G.: Ber. **21**, 1940 (1888).

(256) Majima, R., and Hoshino, T.: Ber. **58**, 2042 (1925).

(257) Majima, R., Unno, T., and Ono, K.: Ber. **55B**, 3854 (1922).

(258) Manske, R. H. F., and Leitch, L. C.: Can. J. Research **14B**, 1 (1936).

(259) Manske, R. H. F., Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **1927, 1.**

(260) Manske, R. H. F., and Robinson, R.: J. Chem. Soc. **1927**, 240.

(261) Marchionneschi, M.: Gazz. chim. ital. **37**, ii, 201 (1907).

(262) Maurer, H., and Moser, E.: Z. physiol. Chem. **161**, 131 (1926).

(263) Mauthner, J., and Suida, W.: Monatsh. **7**, 230 (1886).

(264) Mauthner, J., and Suida, W.: Monatsh. **10**, 250 (1889).

(265) Mazzara, G., and Borgo, A.: Gazz. chim. ital. **35**, ii, 563 (1905/6).

(266) Menitzescu, C.: Ber. **58B**, 1063 (1925).

(267) Menon, K. N., and Robinson, R.: J. Chem. Soc. **1931, 773.**

(268) Michaelis, A.: Ber. **30**, 2809 (1897).

(269) Michaelis, A.: Ber. **30**, 2811 (1897).

(270) Michaelis, A., and Luxembourg, K.: Ber. **26**, 2176 (1893).

(271) Miller, E. J., and Robson, W.: J. Chem. Soc. **1938**, 1910.

(272) Mitsuwa, T., Mori, K., and Kotake, M.: J. Chem. Soc. Japan **58**, 310 (1937).

(273) Montignie, E.: Bull. soc. chim. **51,** 689 (1932).
(274) Morgan, T.: Chem. News **36,** 269 (1877).
(275) Munerati, O.: Compt. rend. **206,** 762 (1938).
(276) Nef, J. U.: Ann. **266,** 72 (1891).
(277) Nelson, V. E.: J. Biol. Chem. **24,** 527 (1916).
(278) Nencki, M.: Ber. **7,** 1593 (1874).
(279) Nencki, M.: Ber. **8,** 722 (1875).
(280) Nencki, M.: J. prakt. Chem. [2] **17,** 97 (1878).
(281) Nencki, M.: J. prakt. Chem. [2] **20,** 466 (1879).
(282) Nencki, M.: Monatsh. **10,** 506 (1889).
(283) Nencki, M., and Berlinerblau, J.: German patent 40,889 (1884).
(284) Nencki, M., and Frankiewicz, F.: Ber. **8,** 336 (1875).
(285) Oddo, B., and Alberti, C.: Gazz. chim. ital. **63,** 236 (1933).
(286) Oddo, B., and Crippa, G. B.: Gazz. chim. ital. **54,** 339 (1924).
(287) Ohta, T.: J. Pharm. Soc. Formosa **51,** December (1938); Chem. Abstracts **34,** 5846 (1940).
(288) Onslow, H.: Biochem. J. **15,** 383 (1921).
(289) Orechoff, A., and Norkina, S.: Ber. **68,** 436 (1935).
(290) Osborne, T. B., and Clapp, S. H.: Am. J. Physiol. **19,** 475 (1907).
(291) Osborne, T. B., and Clapp, S. H.: Z. anal. Chem. **49,** 146 (1910).
(292) Osborne, T. B., and Harris, I. F.: J. Am. Chem. Soc. **25,** 853 (1903).
(293) Oswald, A.: Z. physiol. Chem. **73,** 128 (1911).
(294) Oxford, A. E., and Raper, H. S.: J. Chem. Soc. **1927,** 417.
(295) Pauly, H., and Gundermann, K.: Ber. **41,** 3999 (1908).
(296) Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **115,** 933 (1919).
(297) Perkin, W. H., Jr., and Robinson, R.: J. Chem. Soc. **115,** 967 (1919).
(298) Pfeiffer, P., Angern, O., and Wang, L.: Z. physiol. Chem. **164,** 182 (1927).
(299) Pfulf, A.: Ann. **239,** 215 (1887).
(300) Phisalix, C., and Bertrand, G.: Compt. rend. soc. biol. **45,** 477 (1893).
(301) Phisalix, C., and Bertrand, G.: Compt. rend. soc. biol. **54,** 932 (1902).
(302) Piccinini, A.: Atti accad. Lincei [5] **8,** i, 312 (1899).
(303) Piccinini, A., and Salmoni, L.: Gazz. chim. ital. **32,** i, 246 (1902).
(304) Pictet, A.: Ber. **19,** 1063 (1886).
(305) Pictet, A., and Duparc, L.: Ber. **20,** 3416 (1887).
(306) Plancher, G. Ber. **31,** 1496 (1898).
(307) Plancher, G. Gazz. chim. ital. **28,** i, 409 (1898).
(308) Plancher, G. Gazz. chim. ital. **28,** ii, 349 (1898).
(309) Plancher, G. Gazz. chim. ital. **28,** ii, 389 (1898).
(310) Plancher, G. Atti accad. Lincei [5] **9,** i, 115 (1900).
(311) Plancher, G. Gazz. chim. ital. **30,** ii, 564 (1900).
(312) Plancher, G. Gazz. chim. ital. **31,** i, 280 (1901).
(313) Plancher, G. Atti accad. Lincei [5] **11,** ii, 182 (1902).
(314) Plancher, G., and Battinelli, O.: Gazz. chim. ital. **29,** i, 110 (1899).
(315) Plancher, G., and Bonavia, A.: Gazz. chim. ital. **32,** ii, 426 (1902).
(316) Plancher, G., and Ponti, U.: Atti accad. Lincei [5] **16,** i, 130 (1907).
(317) Polikier, H.: Ber. **24,** 2954 (1891).
(318) Polonowski, M.: Bull. soc. chim [4] **21,** 191 (1917).
(319) Polonowski, M., and Mitzberg, C.: Bull. soc. chim. [4] **19,** 33 (1916).
(320) Polyakova, K.: Masloboĭno Zhirovoe Delo **11,** 452 (1935).
(321) Porcher, C.: Compt. rend. **147,** 214 (1908).
(322) Porcher, C.: Bull. soc. chim. [4] **5,** 526 (1909).
(323) Prud'homme, M.: Bull. soc. chim. [2] **28,** 558 (1877).
(324) Pschorr, R., and Hoppe, G.: Ber. **43,** 2543 (1910).
(325) Pyman, F. L., and Levene, H. H. L.: British patent 382,124 (1932).

(326) Raschen, J.: Ann. **239**, 223 (1887).
(327) Raymond-Hamet, M.: Compt. rend. **187**, 142 (1928).
(328) Reddelien, G.: Ann. **388**, 179 (1912).
(329) Reif, G.: Ber. **42**, 3036 (1909).
(330) Reissert, A.: Ber. **30**, 1030 (1897).
(331) Reissert, A.: Ber. **36**, 4326 (1903).
(332) Reissert, A., and Heller, H.: Ber. **37**, 4378 (1904).
(333) Restelli, E.: Anales asoc. quím. argentina **23**, 58 (1935); Chem. Abstracts **30**, 1054 (1936).
(334) Rettger, L. F.: Am. J. Physiol. **8**, 284 (1903).
(335) Richards, M. B.: J. Chem. Soc. **97**, 977 (1910).
(336) Richter, V. v.: Ber. **16**, 679 (1883).
(337) Robinson, R., and Robinson, G. M.: J. Chem. Soc. **113**, 639 (1918).
(338) Robinson, R., and Robinson, G. M.: J. Chem. Soc. **125**, 827 (1924).
(339) Robinson, R., and Suginome, H.: J. Chem. Soc. **1932**, 304.
(340) Roder, A.: Ann. **236**, 164 (1886).
(341) Romburgh, P. van: Rec. trav. chim. **14**, 65 (1895).
(342) Romburgh, P. van: Proc. Koninkl. Akad. Wetenschappen Amsterdam **13**, 1177 (1911).
(343) Romburgh, P. van, and Barger, G.: J. Chem. Soc. **99**, 2068 (1911).
(344) Sack, J.: Pharm. Weekblad **48**, 307 (1911).
(345) Salkowski, E.: Z. physiol. Chem. **8**, 447 (1884).
(346) Salkowski, E.: Z. physiol. Chem. **9**, 8 (1885).
(347) Salkowski, E., and Salkowski, H.: Ber. **12**, 648 (1879).
(348) Schmitz-Dumont, O.: Ann. **514**, 267 (1934).
(349) Schmitz-Dumont, O., Hamann, K., and Diebold, A.: Ber. **71B**, 205 (1938).
(350) Scholtz, M.: Ber. **46**, 1082 (1913).
(351) Scholtz, M.: Ber. **46**, 2138 (1913).
(352) Schwalbe, C. G., Schulz, W., and Jockheim, H.: Ber. **41**, 3792 (1908).
(353) Segal: Breslauer Äitzliche Zeit. **3** and **4** (1884).
(354) Seka, R.: Ber. **56B**, 2058 (1923).
(355) Simnitzki, S.: Z. physiol. Chem. **39**, 113 (1903).
(356) Smith, S.: J. Am. Med. Assoc. **111**, 2201 (1938).
(357) Smith, S., and Timmis, G. M. J. Chem. Soc. **1930**, 1390.
(358) Smith, S., and Timmis, G. M. J. Chem. Soc. **1932**, 763.
(359) Smith, S., and Timmis, G. M. Nature **133**, 579 (1934).
(360) Smith, S., and Timmis, G. M. J. Chem. Soc. **1934**, 674.
(361) Smith, S., and Timmis, G. M. Nature **136**, 259 (1935).
(362) Smith, S., and Timmis, G. M. J. Chem. Soc. **1936**, 1440.
(363) Soden, H. v.: J. prakt. Chem. [2] **69**, 256 (1904).
(364) Späth, E.: Monatsh. **40**, 351 (1919).
(365) Späth, E.: Monatsh. **41**, 401 (1920).
(366) Späth, E., and Lederer, E.: Ber. **63B**, 120 (1930).
(367) Spica, M., and Angelico, A.: Gazz. chim. ital. **29**, ii, 54 (1899).
(368) Staedel, W.: Ber. **21**, 2197 (1888).
(369) Stedman, E., and Barger, G.: J. Chem. Soc. **127**, 247 (1925).
(370) Steensma, F. A.: Z. physiol. Chem. **47**, 25 (1906).
(371) Stöckly, F.: J. prakt. Chem. [2] **24**, 17 (1881).
(372) Stoll, A.: Science **82**, 415 (1935).
(373) Stoll, A., and Burckhardt, E.: Compt. rend. **200**, 1680 (1935).
(374) Stoll, A., and Hofmann, A.: U. S. patent 2,090,430 (1937).
(375) Stoll, A., and Hofmann, A.: Z. physiol. Chem. **251**, 155 (1938).
(376) Sugasawa, S., Satoda, I., and Yamagisawa, J.: J. Pharm. Soc. Japan **58**, 139 (1938).

(377) TANAKA, Z.: J. Pharm. Soc. Japan **60**, 74 (1940).
(378) TANAKA, Z.: J. Pharm. Soc. Japan **60**, 219 (1940).
(379) THIELE, J., AND DIMROTH, O.: Ber. **28**, 1411 (1895).
(380) TIEDMANN AND GMELIN: *Die Verdauung nach Versuchen*. Heidelberg, Leipzig (1826).
(381) TOMICEK, O.: Chem. Listy **16**, 1, 35 (1922).
(382) TRENKLER, B.: Ann. **242**, 112 (1888).
(383) UNGER, O., AND GRAFF, G.: Ber. **30**, 2389 (1897).
(384) VAN DER LEE, J.: Rec. trav. chim. **44**, 1089 (1925).
(385) VERLEY, A.: Bull. soc. chim. **35**, 1039 (1924).
(386) VOISENET, E.: Bull. soc. chim. [IV] **5**, 736 (1909).
(387) VORLÄNDER, D., AND APELT, O.: Ber. **37**, 1134 (1904).
(388) WALKER, C.: Am. Chem. J. **15**, 576 (1893).
(389) WALKER, C.: Am. Chem. J. **16**, 430 (1894).
(390) WALTER, G.: Ber. **25**, 1261 (1892).
(391) WEEHUIZEN, F.: Pharm. Weekblad **43**, 1209 (1906).
(392) WEERMANN, R. A.: Rec. trav. chim. **29**, 18 (1910).
(393) WEERMANN, R. A.: Ann. **401**, 14 (1913).
(394) WEGL, T.: Z. physiol. Chem. **11**, 339 (1887).
(395) WEGLER, R., AND BINDER, H.: Arch. Pharm. **275**, 506 (1937).
(396) WEISSGERBER, R.: Ber. **43**, 3520 (1910).
(397) WENZING, M.: Ann. **239**, 239 (1887).
(398) WEHMER, C.: *Die Pflanzenstoffe*, 2nd edition, p. 570.
(399) WIDMAN, O.: Ber. **15**, 2547 (1872).
(400) WIELAND, H., HESSE, G., AND MITTASCH, H.: Ber. **64**, 2099 (1931).
(401) WIELAND, H., AND HSING, C. Y.: Ann. **526**, 188 (1936).
(402) WIELAND, H., KONZ, W., AND MITTASCH, H.: Ann. **513**, 1 (1934).
(403) WIELAND, H., AND VOCKE, F.: Ann. **481**, 215 (1930).
(404) WIELAND, H., AND WIELAND, T.: Ann. **528**, 234 (1937).
(405) WILLSTÄTTER, R., AND JAQUET, D.: Ber. **51**, 767 (1918).
(406) WILLSTÄTTER, R., SEITZ, F., AND BRAUN, J. v.: Ber. **58B**, 385 (1925).
(407) WINKLER, S., AND PETERSON, S.: Z. physiol. Chem. **231**, 210 (1935).
(408) WINTERNITZ, H.: Z. physiol. Chem. **16**, 260 (1892).
(409) WISLICENUS, W., AND ARNOLD, E.: Ann. **246**, 334 (1888).
(410) WISLICENUS, W., AND ARNOLD, E.: Ann. **246**, 336 (1888).
(411) WOLFF, L.: Ber. **21**, 123 (1888).
(412) WOLFF, L.: Ber. **21**, 3360 (1888).
(413) ZATTI, C.: Gazz. chim. ital. **19**, 260 (1889).
(414) ZATTI, C., AND FERRATINI, A.: Gazz. chim. ital. **20**, 702 (1890).
(415) ZATTI, C., AND FERRATINI, A.: Ber. **23**, 1359 (1890).
(416) ZATTI, C., AND FERRATINI, A.: Ber. **23**, 2296 (1890).
(417) ZATTI, C., AND FERRATINI, A.: Ber. **23**, 2299 (1890).
(418) ZATTI, C., AND FERRATINI, A.: Gazz. chim. ital. **21**, 19 (1891).
(419) ZIMMERMAN, P. W., HITCHCOCK, A. E., AND WILCOXON, F.: Contrib. Boyce Thompson Inst. **8**, 105 (1936).

NAPHTHENIC ACIDS. II

MANUFACTURE, PROPERTIES, AND USES

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The expanding uses for naphthenic acids have made desirable a compilation of information regarding their occurrence, manufacture, composition, properties, analysis, and uses. The material presented in this paper is illustrative of and organized according to the above topics and has been collected mainly from the patent literature. Since, in a presentation of this type, a detailed discussion of every reference is precluded, a supplementary bibliography has been appended.

The true naphthenic acids are probably normal constituents of all crude oils, in which they occur to the extent of about 0.03 to 3.0 per cent (52, 54, 64, 78, 82). By using an average acid content of 0.1 per cent, Ellis (26) has estimated the potential annual production at 50,000 tons or 12,000,000 gallons. It is rather doubtful whether such a volume of production could be attained, since this would require the treatment of all crude oils produced. It is more probable that certain crude oils or distillates rich in acids will continue to be used as a source of the naphthenic acids.

I. RECOVERY OF NAPHTHENIC ACID

The extraction of naphthenic acid from petroleum products is based upon the formation of sodium naphthenate by the neutralization of naphthenic acid with caustic soda or soda ash. The low concentration of naphthenic acid in crude oil or distillate and the tendency of sodium naphthenate to promote emulsions impose practical difficulties on the apparently simple operation of extracting an acid from a neutral oil. In practice, naphthenic acid is recovered as sodium naphthenate from three different sources. Crude oil or a straight-run distillate is distilled from caustic soda, yielding a residue containing resins, asphalts, oils, and sodium naphthenate. Distillates of the gas-oil and kerosene type are extracted with aqueous solutions of caustic soda or soda ash, yielding an aqueous solution rich in sodium naphthenate. The third source of naphthenic acid is soda sludge, obtained by neutralizing the residual acidity in a sulfuric-acid-treated oil after the acid sludge has been separated. The true naphthenic acids remain unsulfonated during the acid treatment of the oil.

By choosing the sources of naphthenic acid in which it is normally concentrated during the refining operation, one of the difficulties in the recovery operation has been largely overcome. While the difficulty of the formation of emulsions was not so easily eliminated and is associated with sodium naphthenate irrespective of its source, there are distinct differences in the handling of the salt from each of the above sources. In view of these differences the following illustrative methods of recovery of naphthenic acid are presented in relation to the source material.

The alkaline residue from the stills in which oil has been distilled from caustic soda contains resins, asphaltic materials, oil, and sodium naphthenate. Two general methods have been reported for the handling of the still residue: In one, the residue is first diluted with a light oil or solvent before recovery of the sodium naphthenate, while in the other the residue is acidified directly with a mineral acid. Accordingly, Powell (69) dilutes the still bottoms with an oil and extracts the sodium naphthenate from the hot mixture by discharging it on a stream of flowing water. In place of oil, Becker and Sloane (8) use a relatively low boiling naphtha as the diluent for the still bottoms.

A somewhat different scheme has been devised by Andrews and Lauer (4), who extract the still bottoms with 80–95 per cent ethyl alcohol in which the soaps are soluble, but which is not a solvent for the oil. Other low-molecular-weight alcohols, such as methyl and isopropyl, may be used. The choice of alcohol is largely dependent upon its cost.

The direct acidification of alkaline still bottoms with a mineral acid is the simplest method of recovery for naphthenic acids, though acids so prepared often contain oil which must later be removed. Despite the obvious steps involved in the acidification process, several patents have been issued covering minor improvements in the operation. Cook (18) dilutes the still bottoms with about ten parts by weight of water and acidifies the mixture with strong hydrochloric acid. Kaufman and Lauer (46) also record essentially the same process as above, as well as the direct acidification of the alkaline residues with dilute acid. The recovery of naphthenic acid and its simultaneous incorporation into an asphalt composition have been disclosed by Swerissen (85), who acidifies a mixture of alkali naphthenates and asphalt as obtained in still bottoms with sufficient acid just to free the naphthenic acids. The recovery of naphthenic acid and its incorporation into a cutting oil by a unified process have also been recorded by Terrell, Hughes, and Carter (89). The mixture of alkali naphthenates and asphalt is acidified, mixed with an oil of lower boiling point than the asphalt, and distilled. The distillate, containing oil and naphthenic acid, is then neutralized to form a cutting oil. This patent is of further interest in that it illustrates the distillation of naphthenic acids through the medium of a carrier liquid, thereby minimizing decarboxylation.

In general, the removal of oil from alkali-naphthenate-containing still bottoms is incidental to the recovery of the acids. Wunsch (93), however, has devised a process specifically for this purpose. The sludge is dispersed in water, thinned with water, and deëmulsified by the addition of water-soluble compounds, such as alcohols, ketones, and salts, while the temperature is maintained at 90–100°C. The acids may be recovered by acidification of the purified aqueous solution. Experience has shown that this method is quite effective when combined with a solvent extraction of the deëmulsified oil.

The recovery of naphthenic acid under anhydrous conditions has been described by Brunck, Kreutzer, and Boeck (14), who treat the alkaline still bottoms with concentrated (98 per cent) sulfuric acid in the presence of a light solvent for the naphthenic acids. It is claimed that the asphaltic materials resinify and precipitate out along with the alkali sulfates. The acids so obtained may, under certain conditions, be contaminated with oil-soluble sulfonic acids.

II. PURIFICATION OF NAPHTHENIC ACIDS

For many practical uses the naphthenic acids as obtained from petroleum are too impure. The impurities associated with the acids are chiefly oil, phenols, basic nitrogen compounds and, to a minor extent, sulfur-containing compounds. While numerous methods of purification have been suggested, only those which combine relatively simple operations and low cost are of importance commercially. As a rule, simple distillation under reduced pressure yields an acid of sufficient purity for most purposes. Table 1 shows the results obtained on two samples of naphthenic acid distilled commercially.[1] According to these results it appears that single distillations will yield a product of 92 to 93 per cent total acid content and having a pale color. Although distillation yields marked improvement in color and acid number, there is a lesser improvement in odor.

TABLE 1

Properties of original and distilled naphthenic acids

ACID	ORIGINAL		DISTILLED	
	Acid No.	Unsaponifiable	Acid No.	Unsaponifiable
		per cent		*per cent*
A................	256	8.0	263	7.4
B	205	13.5	235	7.9

A distillation process claiming the decolorization as well as deodorization of naphthenic acid has been reported by Ewing (27), who first distills the crude acids and then redistills the distillate from a metallic oxide such as cupric oxide or other non-volatile oxidizing agents to remove phenols and odoriferous materials. Volatile oxidizing agents such as air have also been used, both in the presence and in the absence of catalysts or of non-volatile oxidizing agents (28). The formation of color in naphthenic acids, particularly after distillation, has been attributed to the oxidation of phenols and the above processes are designed to remove them. Merrill and Blount (61) separate phenols and naphthenic acid by means of a selective neutralization with soda ash or caustic soda. This mixture is then distilled with steam to remove the phenols and oils.

Several investigators have reported on the treatment of naphthenic acids with strong sulfuric acid as a method of purification. While it was originally thought that sulfuric acid changed the properties of naphthenic acids even to the extent of sulfonating them (81), later work by Charitschkoff (17, 37), Gurwitsch (37), and Tanaka and Nagai (87) on acids from different sources indicates that the acids remain unattacked. The treatment with sulfuric acid serves to precipitate sludge-forming materials from the naphthenic acids as well as to carry down with the sludge some phenols and basic impurities. In general, sulfuric acid of higher concentration than 85 per cent is miscible with naphthenic acid, though its solubility is markedly decreased by the presence of petroleum solvents. Such solvents decrease the viscosity of the naphthenic acid and facilitate the separation of sludge and acid. Such a process has been reported by Rutherford (77).

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[1] Private communications.

Carr (15) found, however, that sulfuric acid of at least 100 per cent concentration could be used without the difficulty of solubility arising if 0.5 to 5.0 per cent of acid, based on the naphthenic acid, were used. With this quantity sludge separated readily, while if 10 to 15 per cent was employed mutual solubility and no purification resulted. Hendrey (40) prefers to treat partially purified naphthenic acids with about 5 per cent of 98 per cent sulfuric acid and then dilute the mixture to throw out the sludge. The naphthenic acid is then distilled for final purification. A combination treatment of naphthenic acids with sulfuric acid of 50 to 83 per cent concentration and then activated clay has been reported by Ewing (29). A slightly different process has also been suggested by Rabinovich and Osenova (70), who treat a 50 per cent solution of naphthenic acids in gasoline with concentrated sulfuric acid. This solution is then treated with an adsorbing material (clay), after which it is washed with an aqueous solution of zinc chloride or calcium chloride.

III. COMPOSITION OF NAPHTHENIC ACIDS

The commercially available naphthenic acids are at present identified by origin and acid number. The lack of a more definite and scientific classification is probably due to the complexity of the mixtures of acids now known as naphthenic acid and to the absence of information correlating the properties of the acids from different sources. Chemically, the naphthenic acids have been classified (25) as carboxylic acids of the formulas $C_nH_{2n-2}COOH$ and $C_nH_{2n-4}COOH$, with the largest group falling into the former class. Several attempts to demonstrate the presence of cyclohexane derivatives (12, 94, 95) in more than traces indicate that such compounds are probably present in very minute quantities.

In the absence of cyclohexane derivatives the commercial naphthenic acids may be said to be essentially mixtures of small quantities of low-molecular-weight fatty acids and cyclopentane acids. The latter may be considered as the real naphthenic acids. According to Schutze, Shive, and Lochte (83), the products of the index of refraction and density "show that aliphatic acids have products ranging from 1.280 to 1.350 with most values between 1.300 and 1.310, the naphthenic acids range from 1.390 to 1.470 with most values between 1.410 and 1.440, while phenols have *nd* products above 1.500 and hydrocarbon values below 1.300 and usually below 1.280." Temperatures of 20°C.±2° were used. The application of this rule to naphthenic acids from different sources and of different molecular weights is illustrated in table 2.

While the data in table 2 are illustrative, calculations based on additional references give the same results. Data of this character are valuable from a general viewpoint only, however, since they do no more than place the naphthenic acids within a certain group of compounds, the cyclopentane acids. Numerous efforts have been made to determine the structures of some of the major constituents of various naphthenic acids, but with little success. The difficulties involved in such studies are chiefly the location and identification of the type of substituents on the cyclopentane ring, the separation of optical and geometrical isomers, and the determination of the number of carbon atoms between the carboxyl group and the ring. von Braun devised a method for determining whether the carboxyl is primary, secondary, or tertiary by treating the naphthenic ethylamide with phosphorus pentachloride and measuring the chlorine present in the regenerated acid. This method is satisfactory for naphtheneacetic acids, but is inapplicable to homologs of acetic acid. Until further reactions of this type are discovered or until the acids are synthesized, it seems likely that the detailed structures of the acids will remain unknown.

TABLE 2

nd values for naphthenic acids

ACID	SOURCE	nd	REFERENCE
$C_8H_{14}COOH$		1.424	(5)
$C_9H_{16}COOH$		1.423	(5)
$C_{10}H_{18}COOH$	Baku	1.419	(51)
$C_{12}H_{22}COOH$	Nishiyama	1.421	(86)
$C_{13}H_{24}COOH$	Nishiyama	1.427	(86)
$C_{14}H_{24}COOH$	Nishiyama	1.444	(86)
$C_{15}H_{28}COOH$	Nishiyama	1.439	(86)

IV. ANALYSIS OF NAPHTHENIC ACIDS

The present uses for naphthenic acids are based mainly on the relatively high acidity of these compounds. As a result of this, acid number and saponification number are of primary importance. These determinations are carried out in alcohol solution by the usual methods, as is also a determination of the unsaponifiable matter. An improved method for the determination of the total acid content has been proposed by Klotz and Littmann (49). In some cases it is desirable also to determine the phenols present as well as the sulfur content.

V. THE USES OF NAPHTHENIC ACIDS

The unique properties of the metallic soaps of naphthenic acids account for the major uses of the acid at the present time. The alkali soaps are extremely soluble in water, and 50 per cent solutions of sodium naphthenates are now available commercially.[2] These solutions flow freely and show no tendency to gel. Since commercial naphthenic acids have acid numbers of 200 to 300, their molecular weights are of the order of those of the fatty acids and the low viscosity of the aqueous solutions of the alkali salts is quite remarkable. In general, also, the naphthenic acids are somewhat stronger acids than those derived from the fats and oils. The pH of the neutral alkali naphthenates in dilute aqueous solution is of the order of 8 to 9, whereas that of sodium stearate is 10 to 11. The alkali naphthenates have been proposed as both emulsifying and demulsifying agents. Frizell (33), for example, uses sodium naphthenate in the preparation of an emulsifiable oil by mixing four parts of dry sodium napthenate with one part of cresol and then dissolving the product in mineral oil. The oils are used in the treatment of wool, as orchard sprays, and as cordage oils. Alleman (2) proposes the use of sodium naphthenate for the preparation of a similar soluble oil, but uses no cresol. In general, the use of a coupling agent is desirable when the alkali naphthenates are used as emulsifiers. In addition to cresol, illustrated above, rosin and ethylene glycol may be used. Neukom (67) describes the preparation of a soluble oil of this type in which a mixture of sodium naphthenate and rosin is blended with ethylene glycol and this blend is then diluted with mineral oil. Alcohols may also function as coupling agents. Merrill (60) prepares a soluble oil by adding 17 parts of butyl alcohol to 70 parts of 92 per cent sodium naphthenate and then diluting this product with mineral oil. Hendrey (39) uses pine oil as a coupling agent in a soluble oil having the following typical composition:

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[2] Nuodex Products Company.

	per cent
Light lubricating oil	78–82
Sodium naphthenate	14–18
Pine oil	2
Water	2

Where sodium naphthenate alone is used as emulsifying agent, as in cutting oils, a very high percentage is required. Hughes (43) reports the preparation of such a product by mixing essentially dry sodium naphthenate with oil in the ratio of about one part of oil to one part of soap.

The preparation of disinfectants of either the tar-acid-oil or pine-oil type is often accomplished by a hot process in which an aging at elevated temperatures is required to complete the saponification of fatty acids used as emulsifying agents. Because of the increased strength of the naphthenic acids as compared with higher fatty acids, the saponification is greatly accelerated and the aging process may be greatly reduced or entirely eliminated. The formulations given in table 3 are illustrative of the "cold process" type of disinfectants which are readily and completely dispersible in water. The ingredients are added, with agitation, in the order shown and the reaction is complete as soon as the solutions become homogeneous.

The use of alkali naphthenates in the manufacture of asphalt emulsions and road-paving materials has been advocated by Limburg (55) and by Lichtenstern (53). The demulsifying action of alkali naphthenates is usually confined to water-in-oil type emulsions as encountered in petroleum-refining operations. While in most cases alkali naphthenates are reported as the demulsifying agents, other derivatives and mixtures of naphthenic acid and other materials are also used. For convenience all these materials are grouped together. For example, DeGroote (20) in one case uses a sulfonated condensation product between naphthenic acid and an aromatic hydrocarbon; in another case (21), acids, salts, and esters (naphthenic) are said to accomplish the same purpose. In a third patent DeGroote and Wirtel (24) show the use of naphthenic acid derivatives in combination with salts of alkylated naphthalenesulfonic acids, and in a fourth (22) in combination with salts of polysulfonic acids. Other modifications are reported by DeGroote (23), Hendrey and Ebaugh (41), and Mead (57). The use of naphthenic acid esters alone as demulsifying agents has also been reported (19). In the breaking of water-in-oil emulsions the function of the various agents used seems to be one of phase reversal rather than a simple breaking of the two phases, though Wells and Southcombe (92) indicate that certain naphthenates give non-emulsifying oils when incorporated in a lubricating oil.

The use of various metallic naphthenates in lubricating compositions is usually confined to greases and to high-pressure and industrial lubricants. Becker (7) reports a lubricant composed of sodium naphthenate, petrolatum, and still wax.

TABLE 3

Disinfectants and deodorants

INGREDIENTS	PER CENT BY WEIGHT	
Tar acid oil (10 per cent)	70	
Tar acid oil (75 per cent)		70
Naphthenic acid (Acid No. 225)	10	10
Oil-soluble petroleum sulfonate	13	13
Caustic soda (50 per cent)	3.2	3.2
Water	3.8	3.8
Pine oil	67.7	70.6
Oil-soluble petroleum sulfonate	5.8	8.8
Naphthenic acid (Acid No. 225)	19.3	11.8
Caustic soda (50 per cent)	7.2	
Triethanolamine		8.8

Other sodium naphthenate compositions are reported by McKee and Eckert (56) and by Kaufman and Puryear (47).

In place of the alkali naphthenates, those of the alkaline-earth and heavy metals are also employed where a different effect is desired. In the case of castor machine oils, where increased "stringiness", viscosity, or oiliness is desired, various naphthenates have been employed. Frizell (34) describes such a product prepared from aluminum naphthenate dissolved in a mineral oil to which has been added a very small quantity of sodium naphthenate. The addition of the sodium naphthenate is claimed as an improvement over the use of aluminum naphthenate alone, as shown by Rebber (71). In addition to the metallic naphthenates, certain other derivatives have been advocated for the preparation of castor machine oils. Steik (84) uses the reaction product between castor oil and naphthenic acid, while Hagedorn (38) employs cellulose naphthenate in a composition containing either animal or mineral oil. Johnson (44) uses naphthenate esters of glycol ethers for the same purpose.

The addition of metallic naphthenates to crankcase lubricants is said to decrease the sticking of piston rings. There appears to be some doubt as to which naphthenate is the best to use. The Texas Company (90), for example, suggests the use of stannous naphthenate in quantities of 0.05 to 5.0 per cent. Evidence is presented that this compound is superior to zinc, iron, or aluminum naphthenate. Neely (66), on the other hand, states that aluminum, zinc, magnesium cobalt, cadmium, manganese, and tin (stannic?) naphthenates are all effective if used in conjunction with an organic acid. Nickel naphthenate has been advocated by Brandes (11), but the addition of this salt apparently also requires the use of an antioxidant. Substituted phenols were used for this purpose. Where aluminum naphthenate is used to prevent the sticking of piston rings, the basic product seems to be the best. Neely (65) proposes the aluminum dinaphthenate.

The soot-forming tendencies of fuel oil are materially reduced by the addition of metallic naphthenates to the oil. Adams, Ovitz, and Wiley (1) showed that the metallic salts of naphthenic acids or mahogany soaps could effect a reduction of as much as 540°F. in the ignition temperature of the soot. Quantities of added salt varied between 0.5 and 30 per cent, depending upon the frequency and constancy with which the modified fuel was used. A quantitative study of the effect of naphthenates as soot inhibitors has been reported by Fischer and Hulse (30). Table 4 shows the marked reduction in soot formation.

TABLE 4

Effect of naphthenates in fuels on the formation of soot

FUEL	WEIGHT PER CENT OF NAPHTHENATE ADDED	WEIGHT PER CENT OF METAL ADDED AS NAPHTHENATE	UNBURNED CARBONACEOUS MATERIAL (IN PER CENT OF THAT FORMED WHEN BURNING UNBLENDED FUEL)	
			Stack	Total (including tubes)
			per cent	*per cent*
Unblended fuel	0	0	62.6	100.0
Fuel plus zinc naphthenate	0.58	0.047	68.0	94.3
Fuel plus lead naphthenate	0.32	0.078	46.8	83.8
Fuel plus copper naphthenate	1.03	0.062	45.7	79.5
Fuel plus manganese naphthenate	1.27	0.076	38.4	68.0
Fuel plus cobalt naphthenate	1.23	0.074	32.6	67.4
Fuel plus iron naphthenate	1.13	0.068	29.5	62.6

The metallic naphthenates have also found industrial application in the fields of preservatives and driers. As preservatives are included fungicides and insecticides. The use of sodium naphthenate in place of soap for the preparation of oil–cresylic acid emulsions as insecticides has been reported by Jones (45), who claims that such an emulsion has increased stability to calcium compounds. A similar product was also reported by Berry (9). An emulsion of copper naphthenate with mahogany soap oil, alcohol, and water has been patented by Rogers and McNeil (73), who advocate this composition for the treatment of seeds and plants.

Copper and zinc naphthenates are effective insecticides and fungicides, and solutions of these salts in petroleum solvents are available commercially. According to Trevor (91), good protection of wood against dry rot or mildew and termites is secured by the application of copper naphthenate. Protection against a wide variety of wood beetles is obtained through the use of zinc naphthenate. Attention is also called to the fact that sandbags are being treated in Britain with copper naphthenate to prevent their rotting. A six- to eight-fold increase in life was observed.[3] Despite the volume of "popular" information on the mildew-proofing of textiles with copper and zinc naphthenates, little or no quantitative data have been heretofore presented. Illustrative of the effectiveness of zinc naphthenate are the results given in table 5, which were obtained on 6-oz. and 12-oz. duck treated with different quantities of zinc naphthenate and buried for 2 weeks in mildew-infested soil maintained at 60–70°F. and about 25 per cent moisture content. The zinc naphthenate contained 12.5 per cent zinc.

TABLE 5

Effectiveness of zinc naphthenate in mildew-proofing of textiles

DUCK WEIGHT	ZINC NAPHTHENATE	TENSILE STRENGTH			
		Initial		Final	
		Warp	Filling	Warp	Filling
ounces	*per cent*				
6	0	117.0	66.0	Falling apart	
	2			63.0	43.0
	4			105.0	82.7
	6			100.0	85.7
	8			116.0	82.3
12	0	216.0	201	13	12
	2			202.0	190.3
	4			202.9	192.7
	6			214.1	204.3
	8			217.0	212.3

General experience has shown that copper naphthenate is much more effective than zinc naphthenate over an extended period of time. This is best illustrated by the results obtained when the accelerated burial test is continued for 7 weeks. At the end of this time a sample of duck containing 0.5 per cent of zinc as naphthenate was falling apart, while the one containing the same amount of copper remained intact and showed only a trace of staining. A further illustration of the protective action of copper naphthenate is given by experiments on cotton seine twine, the results of which are given in table 6. The treated twine contained 0.5 per cent of copper as naphthenate.

The phenol esters of naphthenic acid (88), as well as the glyceryl esters (50), have also been proposed as insecticides. Naphthenic acid has been used to increase the activity of certain insecticides and fungicides (72) for which a polar solvent is required.

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[3] Private communication.

Perhaps the largest present use for metallic naphthenates is in driers. Because of this application a brief description of the general methods of manufacture is given. In the first method, neutral sodium naphthenate solution is mixed with approximately an equivalent quantity of a water-soluble salt of the metal to be combined as naphthenate. Since most of the precipitated naphthenates are sticky, the reaction may be carried out in the presence of a solvent. After purification of the solvent solution, water and excess solvent are removed by distillation. In some cases the precipitated naphthenate may be purified by washing with water under controlled conditions of temperature and agitation. In the second method of manufacture the naphthenic acid is heated with the oxide or carbonate of the metal to be converted to the naphthenate. Since the general principle of manufacture and use of naphthenate driers are as outlined above, reference should be made to the following table of references for information on specific details (table 7).

TABLE 6

Protective action of copper naphthenate on cotton twine

TIME	TENSILE STRENGTH		LOSS	
	Untreated	Treated	Untreated	Treated
days			*per cent*	*per cent*
0	44.5	47		
7	21.5	44	51.6	6.38
14	3.0	46	93.2	2.25
21	0.0	44	100	6.38
28	0.0	32	100	31.9

TABLE 7

Some patents pertaining to driers

INVENTOR AND REFERENCE	SUBJECT OF PATENT
Alleman (3)	Metallic naphthenates from oil emulsions
Bogdan (10)	Stabilizing metallic naphthenates
Fisher (31)	Reaction of naphthenic acid and oxides
Fisher (32)	Combination of lead and other naphthenates
Gardner and Hodge (35)	Naphthenates of metals of sulfide group
Gerlach (36)	Metallic naphthenates from acetates
Meidert (58)	Flux for fusing metallic naphthenates
Meidert (59)	Combination of wool grease and naphthenate driers
Minich (62)	Stabilizing drier solutions
Minich (63)	Insoluble naphthenate driers
Pohl and Insbeck (68)	Washing precipitated naphthenates with hot water
Roon (74)	Pale naphthenate driers
Roon and Gotham (75)	Manufacture of naphthenate driers
Roon and Minich (76)	Naphthenates as grinding agents

Unlike esters of the fatty acids of comparable molecular weight, naphthenic acid esters are solvent plasticizers for cellulose derivatives. Schneider (80) proposes the use of naphthenic acid esters of ether alcohols, while Kirsthaler and Kaiser (48) and Barrett and Lazier (6) propose the use of esters of naphthenic alcohols.

Some unusual uses for naphthenic acid and some of its derivatives have also been reported. Schladebach and Hähle (79) advocate copper naphthenate in a rust-proofing paint, while Brizzolara (13) uses various naphthenates in promoting the dispersion of carbon black. Hintermaier (42) records the preparation of a naphthenic acid–silicic acid anhydride, and Cassidy (16) combines naphthenic acids with heterocyclic nitrogen compounds.

The foregoing discussion on the uses of naphthenic acids is probably far from complete, since their stability and acid character suggest many possible synthetic operations. As the composition and structure of the acids are more clearly understood, there is a possibility that they will find further application in synthetic organic chemistry as well as in the fields already developed.

REFERENCES

(1) Adams, E. W., Ovitz, F. K., and Wiley, E. G. (to Standard Oil Company of Indiana): U. S. patent 2,141,848 (December 27, 1938).

(2) Alleman, G. (to Sun Oil Company): U. S. patent 1,694,462 (December 11, 1928).

(3) Alleman, G. (to Sun Oil Company): U. S. patent 1,694,463 (December 11, 1928).

(4) Andrews, T. M., and Lauer, C. E. (to The Texas Company): U. S. patent 1,804,451 (June 6, 1925).

(5) Aschan, O.: Ber. **23**, 871 (1890); **24**, 2723 (1891).

(6) Barrett, H. J., and Lazier, W. A. (to E. I. duPont de Nemours and Company): U. S. patent 2,047,664 (July 14, 1936).

(7) Becker, A. E. (to Standard Oil Development Company): U. S. patent 1,552,669 (September 8, 1925).

(8) Becker, A. E., and Sloane, R. G. (to Standard Oil Development Company): U. S. patent 1,785,242 (December 16, 1930).

(9) Berry, A. G. V. (to Trinidad Leaseholds, Ltd.): U. S. patent 2,017,391 (October 15, 1935).

(10) Bogdan, M. (to Shell Development Company): U. S. patents 2,199,828 and 2,199,829 (May 7, 1940).

(11) Brandes, O. L. (to Gulf Research and Development Company): U. S. patent 2,202,826 (June 4, 1940).

(12) Braun, J. von: Ann. **490**, 100 (1931).

(13) Brizzolara, R. T. (to E. I. duPont de Nemours and Company): U. S. patent 2,062,159 (November 24, 1936).

(14) Brunck, R., Kreutzer, A., and Boeck, W. (to Deutsche Gasolin A.-G.): U. S. patent 1,938,513 (December 5, 1933).

(15) Carr, D. E. (to Union Oil Company of California): U. S. patent 2,081,475 (May 25, 1937).

(16) Cassidy, T. A. (to Frank J. Busser and Henry T. Hornidge, as trustees): U. S. patent 1,860,850 (May 31, 1932).

(17) Charitschkoff, K. W.: Neft. Dyelo **1905**, 518.

(18) Cook, L. W. (to The Texas Company): U. S. patent 1,802,336 (April 28, 1931).

(19) DeGroote, M. (to W. S. Barnickel and Company): U. S. patent 1,596,597 (August 17, 1926).

(20) DeGroote, M. (to W. S. Barnickel and Company): U. S. patent 1,596,598 (August 17, 1926).

(21) DeGroote, M. (to The Tretolite Company): U. S. patent 1,940,391 (December 19, 1933).

(22) DeGroote, M. (to The Tretolite Company): U. S. patent 1,940,395 (December 19, 1933).

(23) DeGroote, M. (to The Tretolite Company): U. S. patent 1,940,396 (December 19, 1933).

(24) DeGroote, M., and Wirtel, A. F. (to The Tretolite Company): U. S. patent 1,940,392 (December 19, 1933).

(25) Ellis, C.: *The Chemistry of Petroleum Derivatives*, Volume I, pp. 1062, 1073. The Reinhold Publishing Corporation, New York (1937).

(26) Ellis, C.: *The Chemistry of Petroleum Derivatives*, Volume II, p. 1103. Reinhold Publishing Corporation, New York (1937).

(27) Ewing, F. J. (to Union Oil Company of California): U. S. patent 2,035,696 (March 31, 1936).

(28) Ewing, F. J. (to Union Oil Company of California): U. S. patent 2,035,741 (March 31, 1936).

(29) Ewing, F. J. (to Union Oil Company of California): U. S. patent 2,035,742 (March 31, 1936).

(30) Fischer, H. G. M., and Hulse, S. H. (to Standard Oil Development Company): U. S. patent 2,230,642 (February 4, 1941).

(31) Fisher, G. M. (to Socony-Vacuum Oil Company): U. S. patent 2,071,862 (January 20, 1936).

(32) Fisher, G. M. (to Socony-Vacuum Oil Company): U. S. patent 2,116,884 (May 10, 1938).

(33) Frizell, DeR. (to Union Oil Company of California): U. S. patent 1,582,257 (April 27, 1926).

(34) Frizell, DeR. (to Union Oil Company of California): U. S. patent 1,582,258 (April 27, 1926).

(35) Gardner, R. H., and Hodge, H. G. (to Sinclair Refining Company): U. S. patent 1,740,584 (December 24, 1929).

(36) Gerlach, R. M.: U. S. patent 1,875,999 (September 6, 1932).

(37) Gurwitsch, L., and Moore, H.: *Petroleum Technology*, p. 127. D. Van Nostrand Company, New York (1932).

(38) Hagedorn, M. (to Farbenindustrie A.-G.): U. S. patent 1,882,816 (October 18, 1932).

(39) Hendrey, W. B. (to The Texas Company): U. S. patent 2,058,788 (October 27, 1936).

(40) Hendrey, W. B. (to The Texas Company): U. S. patent 2,072,053 (February 23, 1937).

(41) Hendrey, W. B., and Ebaugh, I. A. (to The Texas Company): U. S. patent 2,014,936 (September 17, 1935).

(42) Hintermaier, A. (to Henkel & Cie): U. S. patent 2,017,000 (October 8, 1935).

(43) Hughes, E. M. (to Sun Oil Company): U. S. patent 1,577,723 (March 23, 1926).

(44) Johnson, J. W., Jr. (to Atlantic Refining Company): U. S. patent 2,173,117 (September 19, 1939).

(45) Jones, P. R. (to Balfour, Guthrie, & Company): U. S. patent 1,502,956 (July 29, 1924).

(46) Kaufman, G., and Lauer, C. E. (to The Texas Company): U. S. patent 1,986,775 (January 1, 1935).

(47) Kaufman, G., and Puryear, O. P. (to The Texas Company): U. S. patent 2,055,795 (September 29, 1936).

(48) Kirsthaler, A., and Kaiser, W. J. (to Henkel & Cie): U. S. patent 2,010,727 (August 6, 1935).

(49) Klotz, J. R. M., and Littmann, E. R.: Ind. Eng. Chem., Anal. Ed. **12**, 76 (1940).

(50) Knight, H. (to Standard Oil Company of Indiana): U. S. patent 1,949,799 (March 6, 1934).

(51) Komppa, G.: Ber. **62**, 1562 (1929).

(52) Kraemer, G., and Böttcher, W.: Ber. **20**, 596 (1888).

(53) Lichtenstern, R.: U. S. patent 1,671,284 (May 29, 1928).

(54) Lidow, A. P.: Seifensieder-Ztg. **32**, 834 (1905).

(55) Limburg, H. (to Patent and Licensing Corporation): U. S. patents 1,984,023 and 1,984,024 (December 11, 1934).

(56) McKee, J., and Eckert, S. B. (to Sun Oil Company): U. S. patent 1,530,386 (March 17, 1925).

(57) Mead, B. (to Standard Oil Development Company): U. S. patent 2,025,766 (December 31, 1935).

(58) Meidert, F. (to I. G. Farbenindustrie A.-G.): U. S. patent 1,976,182 (October 9, 1934).

(59) Meidert, F. (to I. G. Farbenindustrie A.-G.): U. S. patent 2,049,396 (July 28, 1930).

(60) Merrill, D. R. (to Union Oil Company of California): U. S. patent 1,695,197 (December 11, 1928).

(61) Merrill, D. R., and Blount, A. L. (to Union Oil Company of California): U. S. patent 2,000,244 (May 7, 1935).

(62) Minich, A. (to Nuodex Products Company): U. S. patent 2,081,407 (May 25, 1937).

(63) Minich, A. (to Nuodex Products Company): U. S. patent 2,116,321 (May 3, 1938).

(64) Naphtali, M.: Fettchem. Umschau **7**, 149 (1933).

(65) Neely, G. L. (to Standard Oil Company of California): U. S. patent 2,144,078 (January 17, 1939).

(66) Neely, G. L. (to Standard Oil Company of California): U. S. patent 2,163,622 (June 27, 1939).

(67) Neukom, O. W. (to Union Oil Company of California): U. S. patent 2,060,425 (November 10, 1936).

(68) Pohl, F., and Isenbeck, B. (to I. G. Farbenindustrie A.-G.): U. S. patent 1,974,507 (September 25, 1934).

(69) Powell, R. E. (to Standard Oil Development Company): U. S. patent 1,694,280 (December 4, 1928).

(70) Rabinovich, A., and Osenova, T.: Chem. Abstracts **29**, 8304 (1935).

(71) Rebber, L. L. (to Union Oil Company of California): U. S. patent 1,582,227 (April 27, 1926).

(72) Remy, T. P. (to The Texas Company): U. S. patent 2,045,925 (June 30, 1936).

(73) Rogers, F. M., and McNeil, C. P. (to Standard Oil Company of Indiana): U. S. patent 1,679,919 (August 7, 1928).

(74) Roon, L. (to Nuodex Products Company): U. S. patent 2,139,134 (December 6, 1938).

(75) Roon, L., and Gotham, W. (to Nuodex Products Company): U. S. patent 2,113,496 (April 5, 1938).

(76) Roon, L., and Minich, A. (to Nuodex Products Company): U. S. patent 2,180,721 (November 21, 1939).

(77) Rutherford, J. T. (to Standard Oil Company of California): U. S. patent 2,108,448 (February 15, 1938).

(78) Scheller: Petroleum **13**, 731 (1917–18).

(79) Schladebach, H., and Hähle, H. (to I. G. Farbenindustrie A.-G.): U. S. patent 1,983,006 (December 9, 1934).

(80) Schneider, G. (to Celanese Corporation of America): U. S. patent 2,016,392 (October 8, 1935).

(81) Schultz, F.: Chem.-Ztg. **55**, 729 (1908).

(82) Schulz, F.: Chem.-Ztg. **32**, 596 (1908).

(83) Schutze, H. G., Shive, B., and Lochte, H. L.: Ind. Eng. Chem., Anal. Ed. **12**, 262 (1940).

(84) Steik, K. T. (to National Oil Products Company): U. S. patent 2,068,088 (January 19, 1937).

(85) Swerissen, H. T. (to Shell Development Company): U. S. patent 2,026,073 (December 31, 1935).

(86) Tanaka, Y., and Nagai, S.: J. Fac. Eng. Tokyo Imp. Univ. **16**, 1471 (1925).

(87) Tanaka, Y., and Nagai, S.: J. Fac. Sci. Imp. Univ. Tokyo **16**, 117, 183 (1926); **20**, 2832 (1926).

(88) Teichmann, C. F. (to The Texas Company): U. S. patent 2,015,045 (September 17, 1935).

(89) Terrell, H. T., Hughes, E. M., and Carter, P. L. (to Sun Oil Company): U. S. patent 2,056,913 (October 6, 1936).

(90) Texas Company: British patent 510,496 (December 18, 1937).

(91) Trevor, J. S.: Chem. Industries **45**, 661 (1939).

(92) Wells, H. M., and Southcombe, J. E.: U. S. patent 1,319,129 (October 21, 1919).

(93) Wunsch, J. A.: U. S. patent 2,003,640 (June 4, 1935).

(94) Zelinsky, N., and Chuksanov, A.: Ber. **57**, 42 (1924).

(95) Zelinsky, N., and Pokrovskaia, E.: Ber. **57**, 51 (1924).

Supplementary patent references

U. S. patents	
1,286,179	R. E. Humphreys
1,425,882	H. T. Maitland (August 15, 1922).
1,425,883	H. T. Maitland (August 15, 1922).
1,425,884	H. T. Maitland (August 15, 1922).
1,425,885	H. T. Maitland (August 15, 1922).
1,474,933	R. E. Humphreys, F. M. Rogers, and O. E. Bransky (November 20, 1923).
1,482,416	W. O. Snelling (February 5, 1924).
1,529,658	J. McKee and S. B. Eckert (March 17, 1925). Grease.
1,681,657	O. E. Bransky (August 21, 1928).
1,694,461	G. Alleman (December 11, 1928).
1,720,821	S. P. Coleman (July 16, 1929).
1,784,262	R. C. Wheeler and P. W. Prutzman (December 9, 1930).
1,823,615	N. E. Lemmon and F. V. Grimm (September 15, 1931).
1,886,647	S. P. Coleman (November 8, 1932).
1,931,855	G. Alleman (October 24, 1933).
1,931,880	H. F. Angstadt (October 24, 1933).
19,179*	R. C. Wheeler and P. W. Prutzman (May 22, 1934).
1,984,432	J. Robinson (December 18, 1934).
1,998,765	W. B. Logan (April 23, 1935).
2,001,108	C. K. Parker (May 14, 1935). Transformer oil.
2,007,146	F. M. Rogers (July 2, 1935).
2,039,106	R. F. Nelson and L. Zapf (April 28, 1936).
2,093,001	A. L. Blount (September 14, 1937).
2,102,633	K. E. Long (December 21, 1937).
2,138,087	P. E. Burchfield (November 29, 1938).
2,157,766	K. E. Long (May 9, 1939).
2,157,767	K. E. Long (May 9, 1939).
2,157,768	K. E. Long (May 9, 1939).
2,191,449	A. L. Blount (February 27, 1940).
2,205,994	C. C. Towne (June 25, 1940). Manufacture of naphthenates.
2,206,002	A. J. Deutser and R. F. Nelson (January 25, 1940). Manufacture of naphthenates.

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* Reissue.

Supplementary references

CHERCHEFFSKY, N.: *Les acides de naphthe et leur applications.* H. Dunod et E. Pinat, Paris (1910).

NAPHTALI, M.: *Naphthensäuren und Naphthensulfosäuren.* Wissenschaftliche Verlagsgesellschaft, Stuttgart (1934).

NAPHTALI, M.: *Chemie, Technologie, und Analyse der Naphthensäuren.* Wissenschaftliche Verlagsgesellschaft, Stuttgart (1927).

THE CHEMISTRY OF QUINOLINES

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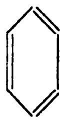
The review is an attempt to deal with the chemistry of quinolines under the following headings: (*a*) reactions which lead to the synthesis of quinolines; (*b*) the reactions of quinolines not dependent upon the presence of substituents; (*c*) the reactions of quinolines dependent upon substituents; and (*d*) the natural occurrence of quinolines.

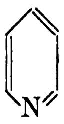
The discovery of quinoline in 1842 by Gerhardt as the result of the drastic decomposition of quinine and of cinchonine antedates Anderson's discovery of pyridine by four years. It was not, however, until Weidel in 1879 and later Ladenburg studied the pyridine bases in detail that the foundations for the chemistry of quinoline and isoquinoline were well and truly laid. The latter was discovered in the quinoline fraction of coal tar by Hoogewerff and van Dorp in 1885. It was largely due to the efforts of these chemists that subsequent work on the isoquinoline alkaloids proved so fruitful in the next century.

The constitution of pyridine follows not only from the many syntheses but analytically from a number of important observations. Reduction adds six hydrogen atoms to pyridine, producing a secondary base,—namely, piperidine, $C_5H_{11}N$. Piperidine is a saturated compound and therefore monocyclic. A variety of methods are available for opening this ring, with the formation of an unbranched chain of five carbon atoms. It is thus possible to write pyridine (II) as a simple derivative of benzene (I) in which a methine group has been replaced by N≡.



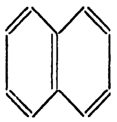
I

Benzene



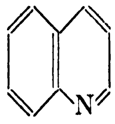
II

Pyridine



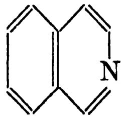
III

Naphthalene



IV

Quinoline



V

Isoquinoline

Just as naphthalene (III) may be regarded as the condensation product of two benzene nuclei, it is possible to regard quinoline (IV) and isoquinoline (V) as benzpyridines, quinoline being benz-2,3-pyridine and isoquinoline being benz-3,4-pyridine.[1] Like the benzene nucleus, the pyridine nucleus possesses aromatic character. The double bonds do not add groups or radicals, and if reaction does occur (except with hydrogen), it is in general one of substitution.

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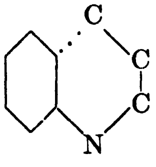
[1] The question of the nature of the unsaturation of the pyridine nucleus and the disposition of the double bonds is strictly analogous to the same problem of the benzene nucleus. No attempt will be made in this review to discuss such problems.

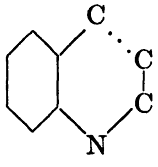
Whether or not the pyridine nucleus is more aromatic in character than the benzene nucleus depends upon the criteria chosen. Certainly it is extremely resistant to a variety of chemical changes, of which oxidation and decomposition by heat alone may be taken as examples. When benzpyridines are oxidized, the benzene nucleus is attacked first. The resultant products are generally pyridinedicarboxylic acids.

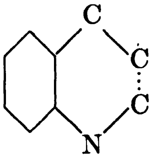
In the following pages an effort will be made to elucidate the salient points in the chemistry of quinolines. No attempt will be made to catalogue all of the compounds. This has been done by Hollins (109) up to the time of the publication of his very excellent book. Further, the naturally occurring alkaloids of the quinoline and isoquinoline group have been ably and exhaustively treated by Henry (105) in the third edition of his book and only casual reference to this subject is deemed desirable. There remains the subject of syntheses and of reactions.

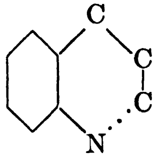
I. SYNTHESES OF QUINOLINE AND OF QUINOLINE DERIVATIVES

Subsequent to the elucidation of its structure, the synthesis of quinoline by Skraup was probably the greatest single impetus to its further study. Not only was quinoline readily available from easily accessible materials, but a legion of substitution products could be as readily obtained. This reaction, which is one of the great classics of organic chemistry, has been the subject of numerous researches into its possible mechanism as well as of many attempts to improve its utility as far as yields and safety are concerned. In general, it is carried out by heating a mixture of an aromatic amine (with one of the ortho positions unsubstituted), glycerol, sulfuric acid, and an oxidizing agent. The last may be the nitro compound corresponding to the amine used, but arsenic acid, ferric oxide, and even picric acid have been recommended. There have been described some thirty procedures by which quinoline or its derivatives may be obtained. Some of these are but slight variations of the others, and all but a few require aniline or a derivative as one of the starting materials. That is to say, the benzene nucleus and the nitrogen of the quinoline are already joined. In these syntheses, therefore, it becomes a problem of ring closure between the nitrogen and the ortho position of the benzene ring, with the addition of the required number of carbon atoms. There are, therefore, only four fundamental syntheses possible, all others being variations of these. The following four partial formulas indicate this graphically. The dotted line indicates the two atoms which must be united to complete the hetero ring. In Type I the ring is to be completed by a union between the γ-carbon atom and the benzene nucleus; in Type II the


I


II


III

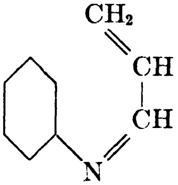

IV

union is to be between the β- and γ-carbon atoms; in Type III the union is to be between the α- and β-carbon atoms; and in Type IV the union is to be effected between the nitrogen atom and the α-carbon atom. There are many syntheses which superficially do not fit into this simple classification. For example, the condensation of *o*-aminobenzaldehyde with ketones introduces carbon atoms α and β in the one procedure and in effect is a combination of Types II and IV. Nevertheless, it is virtually certain that this reaction goes in stages and that the last stage belongs to one of the simple types. The well-known Skraup synthesis is a combination of Types I and IV.

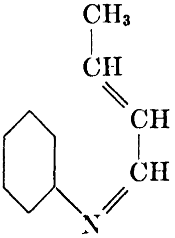
In the following résumé the four types will be discussed separately. In each synthesis where there are two or more stages, only the last will be considered as relegating the synthesis to a definite type. When the last stage is in doubt, a probable mechanism will be assumed. Lastly, a miscellaneous group of syntheses will be detailed.

Type I: The Skraup reaction

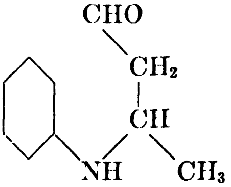
In Type I, ring closure is effected by the elimination of hydrogen from the benzene nucleus and of an atom or group from the γ-carbon atom. A prerequisite is an *N*-substituted aniline containing a chain of three carbon atoms. The latter may be prepared as a separate stage as in the first synthesis of quinoline by Koenigs (137), in which allylaniline was passed over heated litharge. Shortly thereafter, the same author (138) reported the second synthesis of quinoline by heating the condensation product of aniline and acrolein. In both cases the yields were mediocre but the basis for the classical Skraup synthesis (201) was established. In this synthesis aniline, glycerol, sulfuric acid, and an oxidizing agent are heated together. The action of the sulfuric acid on the glycerol yields acrolein, which condenses with the aniline. The nature of this first condensation product is in some doubt. Skraup's original suggestion that a Schiff base (V) is formed is not satisfactory. If it were so, crotonaldehyde, i.e., β-methylacrolein, should yield as an intermediate the Schiff base VI, which on ring closure would give lepidine. The product in the latter case, however, is quinaldine (10), and on this basis Simon (196) has suggested that the intermediate is either compound VII or the Schiff base of the latter. Loss of water or aniline then completes the hetero ring, which, as in Koenigs' synthesis, must still lose hydrogen to become aromatic. The formation of a compound analogous to VII by the



V



VI



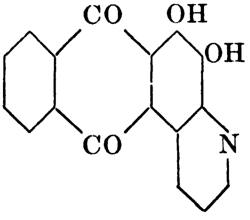
VII

addition of an amine to a double bond is not without analogy. The addition of ammonia to α,β-unsaturated acids frequently gives rise to β-amino acids. Whereas Koenigs in his first synthesis used litharge as an oxidizing agent, Skraup employed nitrobenzene. Knueppel (136) suggested an improvement by employing arsenic pentoxide, particularly when nitroanilines are condensed. Other oxidizing agents such as picric acid (120) and ferric oxide (7) may be mentioned.

The reaction as carried out according to Skraup's procedure frequently proceeds with great violence. Several important modifications have been suggested (37, 41, 47). The one of Cohn (42) which employs, in addition to the Skraup reagents, ferrous sulfate and boric acid, in the writer's experience proceeds smoothly and safely and the yields are as claimed.

The utility of the Skraup reaction is very great. While it is not possible to prepare quinolines substituted in the hetero ring, the record of failures with aromatic amines is conspicuously small. With meta-substituted anilines both the 5- and the 7-substituted quinolines are generally produced if the meta substituent is ortho-para directing. When the substituent is meta directing, only the 5-substituted quinolines result. The same positions are assumed by the meta substituent in the Doebner-Miller synthesis (189).

The reaction takes place with di- and tri-amino-substituted benzenes (176, 203). Mono- and di-carboxylic derivatives of aniline react readily, although a carboxyl group may be eliminated. Thus the products from *m*-aminobenzoic acid (145, 203) and from 3-aminophthalic acid (221) are identical and consist mainly of 7-carboxyquinoline. The naphthylamines react readily. From β-naphthylamine only the angular isomer, namely, 5,6-benzquinoline, is obtained. An attempt to obtain the 6,7-benzquinoline from β-naphthylamine by blocking the α-position with a nitro group or a bromine atom results in the elimination of this substituent, with the formation of the angular isomer (23, 147). The linear isomer is indirectly available through the *ar*-tetrahydro-β-naphthylamine which yields both forms, the linear one predominating (147). An interesting case is that of β-nitroalizarin, from which Prud'homme (183) obtained a blue product by heating with glycerol and sulfuric acid. The product was shown by Graebe (94) to be an anthraquinoline of formula VIII; hence this constitutes the first synthesis of a quinoline derivative without the author being aware that he was to anticipate Skraup.

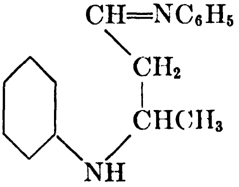


VIII

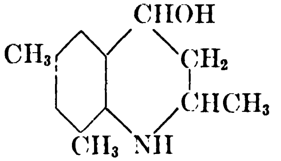
Among other substituted anilines which may be noted there are alkyl-, cyano-, sulfo-, hydroxy-, alkoxy-, halogen-, phenyl-, and other anilines which generally react with facility. Conspicuous by their absence are the reported failures. Among simple amines, *p*-aminoacetophenone (8) yielded no quinoline. Aminoquinolines themselves generally yield the expected condensation product, except for 7-amino-8-methylquinoline (153).

Type I: The Doebner and v. Miller Reaction

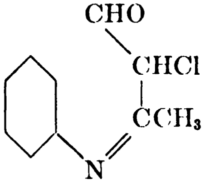
In this synthesis there is the closest analogy to the Skraup reaction. Whereas the latter depends upon the formation of acrolein, the method of Doebner and v. Miller (53) depends upon the intermediate formation of a substituted acrolein, thus yielding quinoline substituted in the hetero nucleus. Cinnamic (54), tiglic (190), and crotonic aldehydes (16) yield 2-phenyl-, 2,3-dimethyl-, and 2-methyl-quinoline, respectively. It is evident that the condensation of aniline with a substituted acrolein to yield quinoline involves the loss not only of water but of two hydrogen atoms as well. Obviously hydrogen is not liberated as gas. From a dimeric form of ethylideneaniline v. Miller obtained a 22 per cent yield of 1,2-dihydroquinaldine by the action of hydrogen chloride. Here a molecule of aniline is liberated and it is probable that the anil (IX) is an intermediate. Eibner (62) has shown that the liberated aniline may be condensed with benzaldehyde *in situ*. The products then are quinaldine or its benzal derivative and the reduction product of benzalaniline,—namely, benzylaniline. Jones and coworkers (61, 92, 116) have shown that the so-called "aldol bases" of v. Miller and Plöchl (162) obtained from 4-*m*-xylidine and acetaldehyde are *cis*- and *trans*-4-hydroxyquinolines of formula X. These compounds are converted by heat or by mineral acids into dihydroquinolines, which by dismutation yield the quinolines and tetrahydroquinolines. The more recent studies of Mills, Harris, and Lambourne (163) have shown that the hydrogen available in the reaction as usually carried out is utilized in the reduction of the anils of the aldehydes which may be present. In the case of acetaldehyde there is formed, in addition to quinaldine and a trace of 6-ethylquinaldine, ethylaniline and *n*-butylaniline, the latter arising from the reduction of the two double bonds in $C_6H_5N{=}CHCH{=}CHCH_3$. The 6-ethylquinaldine is regarded as being derived from *p*-ethylaniline formed as a result of the migration of the ethyl group from the nitrogen.



IX



X



XI

Substances which yield acetaldehyde on treatment with sulfuric acid, such as glycol (53) and lactic acid (178, 224), may be substituted for it without any advantage, however. An interesting variation is the synthesis of 3-chloroquinaldine by Busch and Koenigs (29) by heating aniline hydrochloride and butylchloral hydrate with zinc chloride. Here the intermediate may be figured as having the structure shown in formula XI, and no hydrogen is available for reduction.

With meta-substituted anilines the ring closure takes place in the two possible ways (49), as in the Skraup synthesis, the 5-derivative predominating. In an earlier study Rist (188), however, claimed that the 7-derivative was always obtained.

The reaction is also capable of being extended to a mixture of aldehydes which condense in the course of the reaction to yield a substituted acrolein, and here the condensation of aniline, propionaldehyde, and methylal (161) to yield 3-methylquinoline deserves special mention. Most of the other recorded cases are ones in which benzaldehyde or a derivative is condensed in conjunction with an aliphatic aldehyde, and the products are 2-phenylquinolines (160).

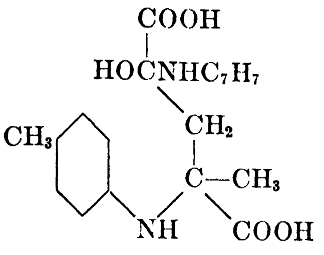
In general, the substituted anilines which yield quinolines in the Skraup synthesis also yield quinolines in the Doebner and v. Miller modification. Failures again are conspicuously absent, although *m*-phenetidine is an example (24). Whereas *p*-aminoacetophenone failed in the Skraup reaction, it does not do so here (8). While aniline reacts normally with acrolein, *p*-toluidine yields no quinoline (152).

Type I: Modifications of the Doebner and v. Miller reaction

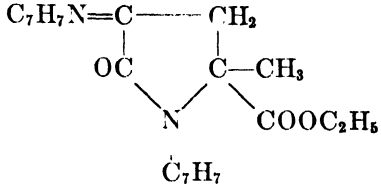
Under this heading may be classed a number of syntheses in which the precursors of the substituted acrolein are not necessarily aldehydes (although one of the reactants frequently is), but their derivatives such as pyruvic acids, ketones, β-ketonic esters, and β-diketones. Expressed in general terms, the reaction involves the condensation of two compounds, $RCOCH_2R'$ and $R''COR'''$ to yield $RCOCR'{=}CR''R'''$, which then reacts with an aromatic amine to yield $RCOCHR'CR''R'''NHAr$. Ultimate ring closure occurs under a variety of experimental conditions. Doebner (51) heated an alcoholic solution of aniline, pyruvic acid, and acetaldehyde and obtained quinaldine-4-carboxylic acid,—the aniluvitonic acid of Böttinger (22). Simon and Mauguin (200) obtained excellent yields by bringing together in cold benzene or chloroform a mixture of α-naphthylamine, benzaldehyde, and ethyl pyruvate. Hydrogen, of course, is not liberated. It is utilized in the reduction of some reactant or intermediate (35, 36).

Some attention has been paid to the mechanism of the condensations involving pyruvic acid. Borsche (25, 26) showed that benzalaniline in boiling alcohol first forms an addition compound with pyruvic acid, $C_6H_5NHCH(C_6H_5)CH_2COCOOH$, which easily yields 2-phenylquinoline-4-carboxylic acid.

Simon (197, 198) studied the reaction between *p*-toluidine and pyruvic acid and the ester. With pyruvic acid an intermediate is formed which is regarded as having the structure shown in formula XII. When heated with water it is converted into 6-methylquinaldine. When *p*-toluidine is condensed with ethyl pyruvate, the main product is not XII but XIII. The latter is obviously



XII



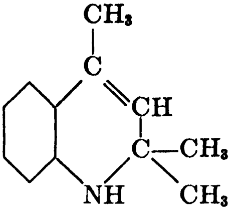
XIII

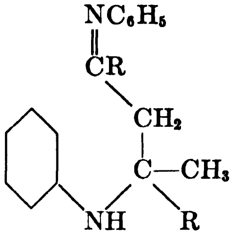
derived from the ester of XII by loss of ethyl alcohol and water and as a derivative of diketopyrrolidine will not yield a quinoline.

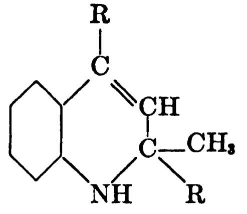
The work of Borsche (25, 26) has extended these observations to the condensation products of a number of arylamines, pyruvic acids, and aldehydes. In most cases involving substituted pyruvic acids, ring closure occurs between the nitrogen and the carboxyl, yielding a diketopyrrolidine which reacts with another molecule of amine forming an anil. The yields of quinoline were in general poor or nil. Only in the case of β-naphthylamine were the quinolines obtained in moderate amounts. The recorded failures with unsubstituted pyruvic acid are comparatively few. Arsanilic acid (115), *m*- and *p*-aminobenzoic acids (52), and the nitroanilines (3) failed to yield quinolines. Most of the other failures are recorded in attempts to use formaldehyde as the second ketonic component, although it has been utilized successfully in a number of cases (26). Aromatic aldehydes react most readily and the α-arylcinchoninic acids or atophans find some use in medicine. A large number of patents covering these substances have been granted

The reaction with an arylamine in which the second molecule of an aldehyde is substituted by that of a ketone was first described by Reed (185), who succeeded in condensing β-naphthylamine with acetaldehyde and acetone. Beyer (16) was the first to synthesize lepidine by this procedure from aniline, formaldehyde, and acetone. The reaction is of wide application and yields a quinoline derivative in which the γ-substituent is supplied by the alkyl of the ketone. Of special interest is the condensation of aniline with chloroacetone and methylal (180). It is remarkable that the chlorine is not eliminated and appears in the 3-chlorolepidine, in spite of the fact that two hydrogens must be removed to obtain the final product.

The fact that one of the molecules of the aldehydes can be replaced by a ketone leads logically to an attempt to realize the condensation in which both molecules of aldehyde are replaced by two molecules of a ketone. In other words, aniline, when condensed with two molecules of acetone, may be expected to yield a substance of formula XIV, that is, a dihydroquinoline from which hydrogen cannot be eliminated to yield a quinoline. Engler and Riehm (71) heated aniline hydrochloride with acetone in the presence or absence of condensing agents and obtained 2,4-dimethylquinoline, together with a gas which proved to consist of methane. So great is the tendency of the hetero ring to become aromatic that not only methane is evolved but other hydrocarbons as well.


XIV


XV


XVI

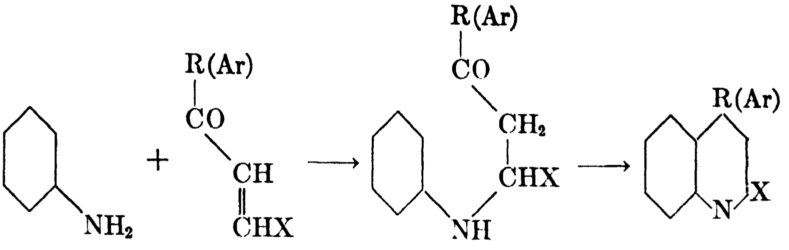
Knoevenagel and his coworkers (124, 126, 129), in their studies of ketone anils, have shed some light on the course of the condensation. The anils of acetone, of methyl ethyl ketone, and of acetophenone give excellent yields of 2,4-disubstituted quinolines when heated with hydrogen chloride at 180–200°C. It is assumed that an intermediate (XV) is formed from 2 moles of the anil which by loss of aniline yields XVI. When R in formula XVI is phenyl, benzene is ultimately eliminated in preference to methane and the main product is 2-methyl-4-phenylquinoline. Heat alone, or heating with phosphorus and hydrogen iodide, gives excellent yields. Mesityl oxide may replace the acetone and even *N*-methylaniline yields a quinoline in the usual manner, the *N*-methyl group being eliminated.

In a limited number of cases involving β-naphthylamine (but not aniline), an aromatic aldehyde, and ethyl oxaloacetate (i.e., carbethoxypyruvic ester), it has been possible to synthesize quinoline derivatives. Simon and Conduché (199) obtained a normal condensation product from benzal-β-naphthylamine and oxalacetic ester. This, on treatment with concentrated sulfuric acid, yielded a dihydroquinoline which readily lost hydrogen, yielding 2-phenyl-β-naphthoquinoline-3,4-dicarboxylic ester. The reaction is further limited, as is the Doebner pyruvic acid synthesis, by the tendency to form diketopyrrolidines or their anils.

Of interest is a synthesis by Halberkann (96), in which *p*-anisidine was condensed with oxalacetic ester alone, yielding 6-methoxycarbostyril-4-carboxylic acid. An analogous and very ingenious synthesis was achieved by Thielepape (219), in which *N*-methylacetanilide was first condensed with ethyl oxalate in the presence of sodium ethylate. The semi-amide of oxalacetic ester thus produced gave a quantitative yield of 1-methylcarbostyril-4-carboxylic ester on treatment with sulfuric acid.

Analogous to the reaction studied by Beyer (16) is one involving α,β-unsaturated ketones. Here the mechanism presumably involves the addition of the arylamine to the double bond, yielding a substance of formula XVII.

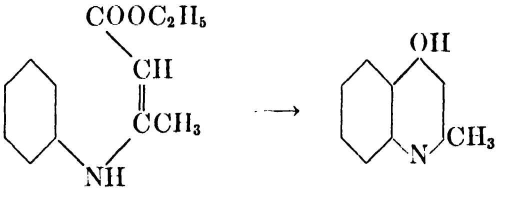
In the case where X and R(Ar) were carboxyl and phenyl respectively, i.e., benzoylacrylic acid, Koenigs and Meimberg (140) obtained 4-phenylquinoline-2-carboxylic acid (XVIII). Blaise and Maire (19), by condensing vinyl ethyl ketone or β-dichloroethyl ketone with aniline, obtained a substance of formula XVII (R = C_2H_5; X = H), which on heating with aniline hydrochloride yielded 4-ethylquinoline. Vinyl propyl ketone and aniline yield 4-propylquinoline and the reaction also goes with anthranilic acid.



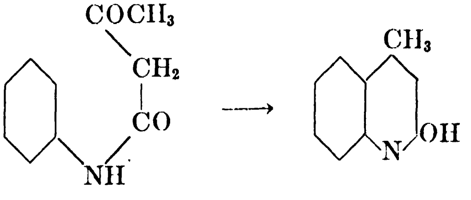
XVII XVIII

Type I: Condensations involving derivatives of β-diketones

Ethyl acetoacetate reacts with aniline, yielding either β-phenylaminocrotonic ester, $C_6H_5NHC(CH_3){=}CHCOOC_2H_5$ (XIX) or acetoacetanilide, $CH_3COCH_2CONHC_6H_5$ (XX). In the cold a nearly quantitative yield of the former may be obtained, whereas at the boiling point the latter is obtained almost exclusively (73, 135), although in only moderate yields. The former on heating to about 240°C. and the anilide on treatment with sulfuric acid yield quinoline derivatives. The reaction was first observed by Conrad and Limpach (46), who prepared 4-hydroxyquinaldine (XXI) and a number of 4-hydroxyquinolines by this method. The reaction proceeds with formylacetic esters and with benzoylacetic ester (132), yielding 2-phenyl-4-hydroxyquinoline. Acetylacetone (44, 45) and aniline yield 2,4-dimethylquinoline, and aroylacetones yield 2-methyl-4-arylquinolines (12, 15). The reaction is of considerable applicability, a number of arylamines having been substituted for aniline. It fails, however, with *p*-phenylenediamine (154) and yields an aminoquinoline with *m*-phenylenediamine (154).



XIX XXI
4-Hydroxyquinaldine



XX XXII
4-Methylcarbostyril

The preparation of 4-substituted carbostyrils was first investigated by Knorr (132, 133) and more recently by Ewins and King (73), who obtained excellent yields of 4-methylcarbostyril (XXII).

Oxalacetic ester (96), acetonedicarboxylic ester (14), and other β-ketonic compounds have been used with a variety of arylamines.

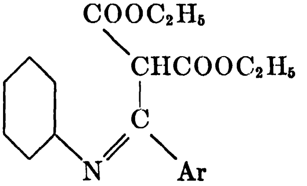
Malonic acid, being in effect a β-diketonic compound, has also been condensed with aniline to yield a quinoline derivative. The semi-anilide of malonic acid on treatment with phosphorus pentachloride yields 2,4-dichloroquinoline, presumably by way of 2,4-dihydroxyquinoline (191). The reaction has found only limited application.

The syntheses of the hydroxyquinolines here typified do not involve the elimination of hydrogen.

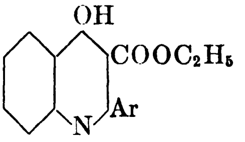
Type I: Miscellaneous syntheses

Under this heading may be grouped a number of quinoline syntheses which, though of limited application, are adaptable to the preparation of compounds difficultly accessible by other routes.

Aromatic imino-chlorides, ArN=CClAr, condense with sodiomalonic ester, yielding compounds of formula XXIII which on heating lose ethyl alcohol with the formation of 2-aryl-3-carbethoxy-4-hydroxyquinolines (XXIV) (117, 118, 119).

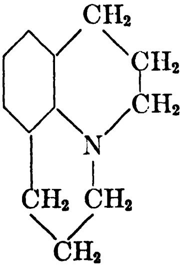


XXIII

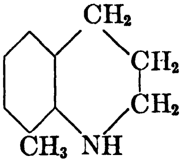


XXIV

Julolidine (XXV) is a tertiary tetrahydroquinoline obtained by Pinkus (181) by heating formanilide with trimethylene chlorobromide. The formyl derivative of *o*-toluidine yielded 8-methyltetrahydroquinoline (XXVI). Tetrahydroquinoline was obtained by Rindfusz and Harnack (187) by treating the con-



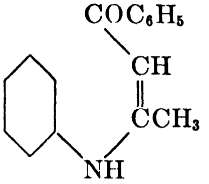
XXV
Julolidine



XXVI

densation product of aniline and trimethylene chlorohydrin with phosphorus pentoxide. From dichlorohydrin and formanilide Bamberger and Kitschelt (5) obtained a complex mixture containing some quinoline as well as skatole.

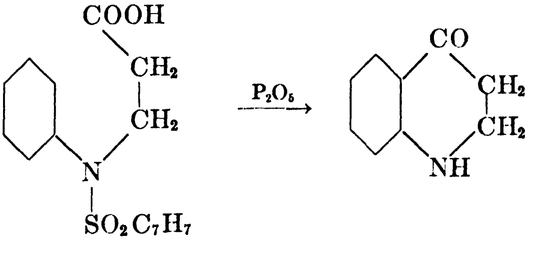
Reminiscent of the reaction of unsaturated ketones with aniline (*vide supra*) is one discovered by Spallino and Salimei (206). Acetanilide when heated with acetophenone and zinc chloride yielded 4-phenylquinaldine, presumably by way of XXVII.



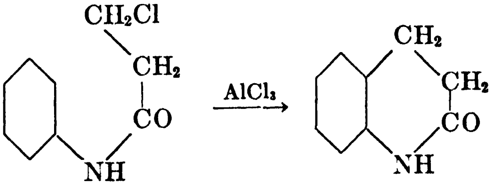
XXVII

Pictet and Barbier (175) had already condensed acetophenone with formanilide. Although an intermediate similar to XXVII is possible, the ultimate product was 2-phenylquinoline. In these reactions the elimination of hydrogen is not necessary.

There remain to be mentioned two syntheses of tetrahydroquinoline derivatives which are of interest in that they virtually amount to a modified Friedel–Crafts reaction. Clemo and Perkin (39, 40) prepared 4-ketotetrahydroquinolines by treating the toluenesulfonyl derivative of β-arylaminopropionic acids with dehydrating agents, the toluenesulfonyl group being eliminated during the reaction or later by acid hydrolysis.



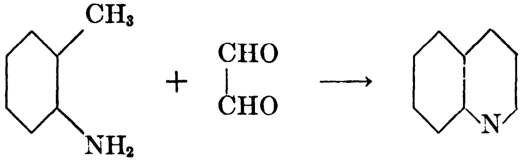
Mayer, van Zütphen, and Philipps (155) developed a general method for preparing dihydrocarbostyrils from the arylamides of β-chloropropionic acid. Aluminum chloride was used to close the ring, and in the twenty-one cases cited the yields were mostly good.



Type II syntheses

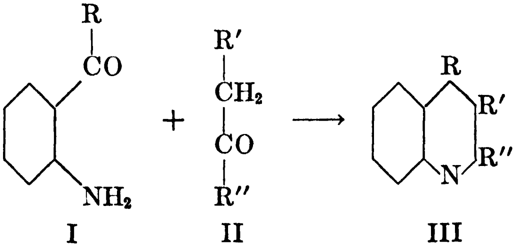
The reactions described under this heading lead to the synthesis of the hetero ring by a union of carbon atoms 3 and 4. There is frequently a certain element of ambiguity as to the mechanism of these processes. In many cases a reaction involving ring closure by union between the nitrogen atom and the 2-carbon atom is also a feasible one. Nevertheless, the latter type is well recognized as a special one where no other mechanism is possible, and those will be considered under Type IV.

Generally speaking, the reactions of Type II necessitate the condensation of an ortho-substituted arylamine with a compound containing two or more carbon atoms. The amino group first condenses with some reactive substance, yielding a product which simultaneously or by a separate condensation eliminates water to form the hetero ring. The simplest example is the synthesis of quinoline from *o*-toluidine and glyoxal in some 35 per cent yield by heating with sodium hydroxide to 150°C. (141).

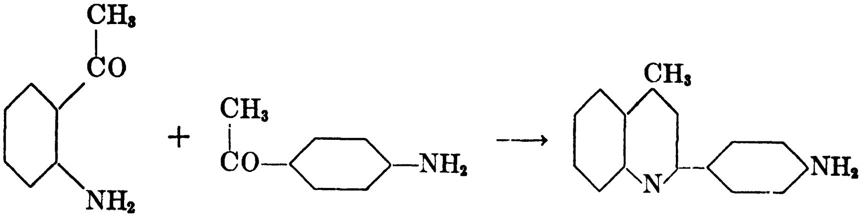


The reaction is not general and fails with benzil (142), although with ethyl pyruvate in the presence of alcoholic zinc chloride Kulisch (142) obtained a compound which was almost certainly 3-hydroxyquinaldine. The Madelung synthesis of indoxylic acid (149), i.e., by heating oxalyl-*o*-toluidine with sodium ethylate, yields a small amount of 2,3-dihydroxyquinoline.

Perhaps the most important type of reaction in this section is the one involving an ortho-amino aroyl compound (I) condensed with a carboxyl compound containing an α-methylene group (II), thus yielding a fully aromatic hetero



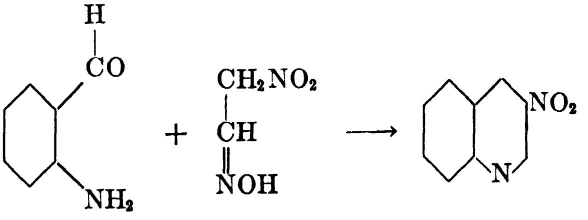
ring (III). The earliest example of this type of condensation is the synthesis of flavaniline by heating acetanilide with zinc chloride. The constitution of this substance and the mechanism of its synthesis were, however, elucidated by Besthorn and Fischer (13) only after Friedlaender (84) in 1882 obtained quinoline by condensing *o*-aminobenzaldehyde with acetaldehyde in the presence of sodium hydroxide. (R, R′, and R″ = H in formulas I, II, and III.) Propionaldehyde condenses with *o*-aminobenzaldehyde to yield a compound which on distillation loses water and forms 3-methylquinoline (228). When acetanilide is heated with zinc chloride, the acetyl group migrates to the ortho and para positions and the aminoacetophenone thus formed yields flavaniline (IV) (13).



IV
Flavaniline

Two molecules of *o*-aminoacetophenone can be condensed by heating with formic acid, yielding an *o*-aminophenylquinoline (18).

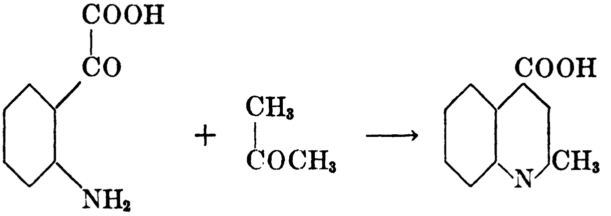
The Friedlaender synthesis is of special importance because of the wide choice of the groups R, R′, and R″, in addition to the fact that the benzene ring may carry a diverse number of substituents. R may be H (*o*-aminoaldehydes), CH_3 and C_6H_5 (*o*-aminoketones), COOH (isatic acids), or OH (anthranilic acids). R′ may be H, alkyl, aryl, NO_2, SO_3H, SO_2Ar, $COOH(C_2H_5)$, CN, $COCH_3$, COC_6H_5, OH, and other groups. R″ may be H, alkyl, aryl, OH, CH=NOH, COOH, etc. It is evident, therefore, that aldehydes, ketones, ketonic esters, acids, nitriles, and other compounds containing at least one carboxyl group may be condensed with aminoaryl compounds containing an ortho-carbonyl group. Failures to effect a quinoline synthesis are exceptional. In the case of *o*-aminobenzaldehyde, the reaction fails with desoxybenzoin (223). Of special interest is the synthesis of β-nitroquinolines by condensations involving methazonic acid, HON=CHCH=NOOH, which reacts as the oxime of nitroacetaldehyde (222, 223). A large number of β-nitroquinolines have been recorded in the patent literature.



β-Nitroquinoline

Diacetyl condenses with two molecules of *o*-aminobenzaldehyde, yielding α,α′-diquinolyl (205). The methyl group of methyl alkyl ketones, CH_3COCH_2R, takes the α-position in the resulting quinoline (67). The condensations with *o*-aminobenzaldehyde are generally brought about by dilute alkali, by heat alone (228), or by means of piperidine (211).

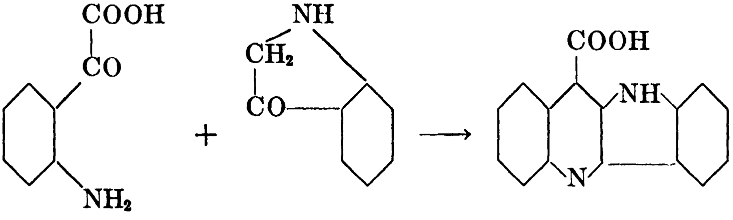
The extension of the Friedlaender synthesis to isatic acid (V) is due to Pfitzinger (172) who substituted this substance for the *o*-aminobenzaldehyde. Thus the condensation with acetone yields aniluvitonic acid (VI). The isatic acid is obtained by heating isatin with aqueous sodium hydroxide and is used directly. With aldehydes the condensation proceeds normally, except that resinification of the aldehyde by the strong alkali may occur. The oximes of the aldehydes react as readily and do not resinify in the presence of alkali (173). The β-oxime of isatin is then obtained as a by-product.



V
Isatic acid

VI
Aniluvitonic acid

The earliest example of the Pfitzinger reaction is the formation of "flavindin," a substance obtained by the over-reduction of indigo (195). Its constitution (quindoline-5-carboxylic acid) (VIII) and its mode of formation were elucidated as follows (75, 167): The reduction of the indigo yielded a mixture of isatin, which hydrolyzed to isatic acid, and indoxyl (VII) and these in the presence of the alkali condensed to yield the flavindin.



VII
Indoxyl

VIII
"Flavindin"

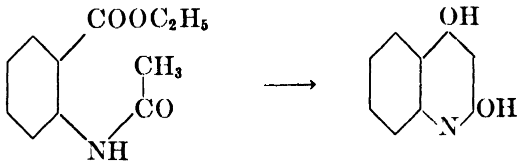
Although acetaldehyde failed to yield cinchoninic acid, the oxime did so (173). Recorded failures in other cases are rare. Levulinic acid, which does not yield a quinoline derivative with *o*-aminobenzaldehyde, reacts normally with isatic acid (70). Acids yield carbostyrils (96).

v. Walther (225) in 1903 introduced an important modification of the Friedlaender–Pfitzinger synthesis. The imino-nitriles of the type $NH{=}CRCH_2CN$ (i.e., dinitriles) react like the corresponding ketones and with isatic acid yield 2-alkyl-3-cyanocinchoninic acid. The reaction has been extended to a number of amino-nitriles in conjunction with *o*-aminobenzaldehyde and with *o*-aminoacetophenone (158). The reaction fails with isatin, yielding instead a complex dihydropyridine (225). This would indicate that the first phase of the condensation is one involving the amino and the imino groups, yielding an arylimino compound through loss of ammonia; this compound subsequently loses water with the formation of the heterocyclic ring. The mechanism of Borsche and Jacobs (27) to account for the condensation of isatin with malonic acid to yield 2-hydroxycinchoninic acid involves the primary condensation between the carboxyl of the isatin and the methylene of the malonic acid. Equally reasonable is the primary formation of a malonylisatin, followed by hydrolysis to malonylisatic acid and ultimate loss of water (and carbon dioxide under the conditions used by them).

In many cases anthranilic acid, when condensed with aldehydes, yields the 8-carboxyquinolines,—the normal product of the Doebner–Miller reaction. Niementowski (166), however, discovered that in some cases the Schiff base of anthranilic acid and an aldehyde (heptaldehyde) (165) yield a small amount of 3-alkyl-4-hydroxyquinoline when heated to 200°C. Acetophenone and anthranilic acid yield 2-phenyl-4-hydroxyquinoline (166). Acetoacetic ester with anthranilic acid yields the 8-carboxycarbostyril by the usual Knorr reaction, but when the position ortho to the amino group is occupied by a methoxyl group, the reaction yields 2-methyl-3-carbethoxy-4-hydroxy-8-methoxyquinoline (86).

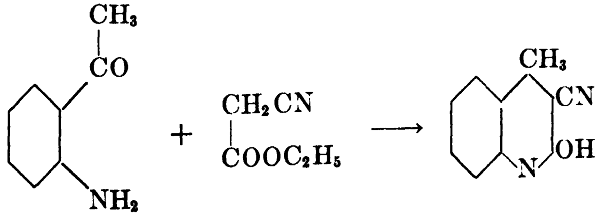
Methazonic acid first forms an anil, which on heating with acetic anhydride yields 3-nitro-4-hydroxyquinoline (59). Benzoylacetic ester yields a complex mixture (166).

An application of the Dieckmann reaction to the synthesis of a quinoline derivative is the conversion of ethyl acetylanthranilate into 2,4-dihydroxyquinoline by treatment with sodium, although the yield in the writer's experience is low (57).

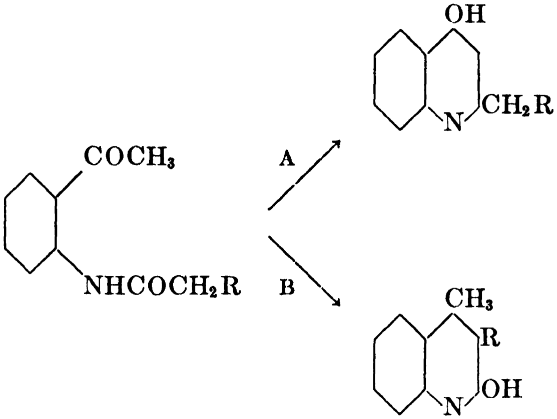


Ethyl acetylanthranilate   2,4-Dihydroxyquinoline

The important reaction by which 4-alkyl (and aryl) derivatives of quinoline may be readily obtained from *o*-acylanilines was extensively studied by Camps (30, 31, 32, 33), who recorded his well-planned experiments in a series of papers. The reaction was discovered by Guareschi, who obtained 2-hydroxy-3-cyano-4-methylquinoline by condensing *o*-aminoacetophenone with ethyl cyanoacetate.



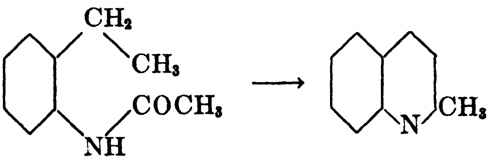
The reaction is strictly analogous to the syntheses involving *o*-aminobenzaldehyde. It offers the advantage that a wide choice of substituents can be introduced into positions 3 and 4, although the 2-position in these cases carries a hydroxyl group. The reaction has been carried out with *o*-amino derivatives of benzophenone, acetophenone, propiophenone (230), benzoylacetic ester (31), and benzoylcarbinol (31). With esters of aliphatic acids two reactions occur simultaneously, the intermediate acylamino compounds forming the ring in two ways. Since the carbostyrils are non-basic, they may be easily separated from the 4-hydroxy compounds which are soluble in acids. When R is C_6H_5, CN, $COCH_3$, COC_6H_5, or $COOC_2H_5$, the reactivity of the hydrogen atoms in the methylene group is greater than that of those in the methyl group and only carbostyrils are obtained. The condensation is brought about by heating the ester with the amino compound, and the yields are good. It is to be observed that reaction A above is an example of ring closure between atoms α and β, that is, Type III.



Type III syntheses

The number of syntheses which may be classed as being of Type III is small and of little general utility. The most important examples are, in fact, those described in the last section, which arise from the fact that the Camps synthesis can take either of two courses.

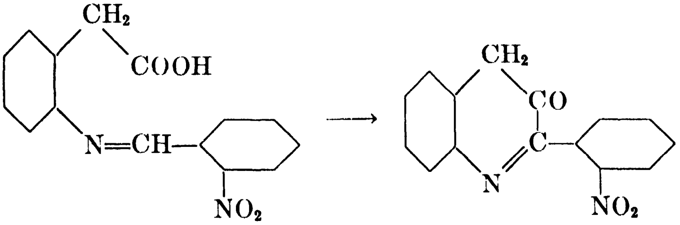
When acetylethylaniline is heated with zinc chloride, a mixture of *o*- and *p*-ethylacetanilides is formed. The former is regarded as giving rise to the small amount of quinaldine that is obtained (177).



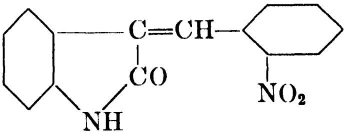
Quinaldine

Acetylmethylaniline, however, yields quinoline under the same conditions (179) and obviously the reaction cannot be formulated in the same way. A more authentic example of ring closure of Type III is one recorded by Gabriel and Löwenberg (89), which by an involved series of reactions led to a complex 4-ketotetrahydroquinoline. Equally involved is the formation of a quinoline derivative from *N*-benzoyltetrahydrocarbazole (170), and the original papers should be consulted. Only slightly less complex is the synthesis of 2,3-dimethyl-6,7-methylenedioxyquinoline from *o*-nitrosafrole (81).

Neber (164) claimed the ring closure of I to yield II, but Kliegl and Schmalenbach (123) showed that the compound formulated as II was *o*-nitrobenzaloxindole (III).



I II

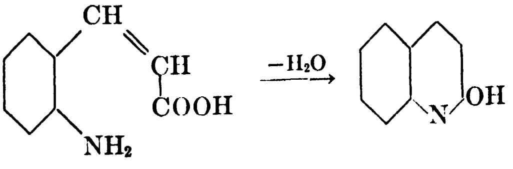


III

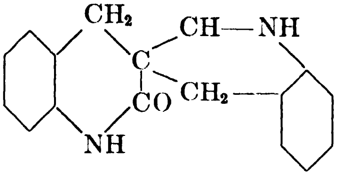
Type IV syntheses

The reactions classed under Type IV involve ring closure between the nitrogen atom and the β-carbon atom by the loss of water or its equivalent. The ortho-substituting group must therefore consist of a chain of at least three carbon atoms, the end one carrying a reactive group.

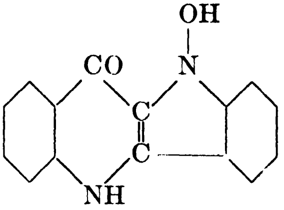
In this section belongs what is probably the first synthesis of a quinoline derivative, namely, carbostyril. It was obtained by Chiozza (34), by reducing *o*-nitrocinnamic acid with ammonium sulfide, the aminocinnamic acid being the intermediate.



The reaction has been the subject of numerous later investigations. A variety of reducing agents has been used (93, 220). The *o*-aminocinnamic acid may be obtained from the *o*-chloro acid by the action of ammonia (159). There seem to be no particular limitations which restrict the utility of the reaction except the accessibility of suitable *o*-nitro- or *o*-amino-cinnamic acids. The reduction with specific reagents frequently yields a 1-hydroxycarbostyril, but further reduction eliminates the hydroxyl group (85, 104, 216). The dihydrocarbostyrils result from the reduction of *o*-nitrodihydrocinnamic acids (65). The preparation of 1-aminodihydrocarbostyril from *o*-hydrazino-β-phenylpropionic acid has been described by Fischer and Kuzel (78). The reduction of di-(*o*-nitrobenzyl)-malonic ester yields a spiro-carbostyril (I) (146), whereas heating with caustic soda results in the loss of the carbethoxyl groups and an intermolecular oxidation-reduction, with the ultimate formation of a substance which has been given formula II and is therefore a quindoline derivative (74).



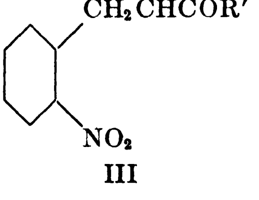
I



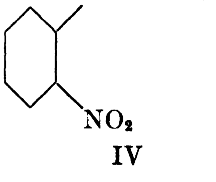
II

o-Aminophenylpropiolic acid does not spontaneously yield a quinoline derivative, but in the presence of sulfuric acid it yields 2,4-dihydroxyquinoline (3). In this reaction *o*-aminobenzoylacetic acid is a probable intermediate. When dilute hydrochloric acid is used, the condensation product is 4-chlorocarbostyril (3, 4). The synthesis of 5-methylquinoline is of interest, in that it cannot be conveniently obtained by any of the methods thus far described. It was obtained by Gabriel and Thieme (90) from 2-nitro-6-methylbenzoylmalonic ester by reduction with phosphorus and hydrogen iodide to 2,4-dihydroxy-5-methylquinoline, which was ultimately reduced to 5-methylquinoline *via* the 2,4-dichloro compound.

An important extension of the carbostyril synthesis is one involving, not the cinnamic acids or their dihydro derivatives, but the β-(*o*-nitroaryl) ketones (III) and their unsaturated derivatives, the *o*-nitrobenzal ketones (IV).



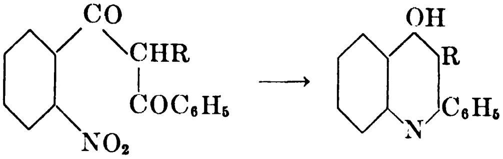
III



IV

Jackson (111) obtained tetrahydroquinaldine by reducing *o*-nitrobenzylacetone, whereas the reduction of *o*-nitrobenzalacetone yielded quinaldine (56). The reduction of *o*-nitrobenzalpyruvic acid (formula IV, R = H and R′ = COOH) yields, not the expected quinaldinic acid, but its 4-hydroxy derivative (99). Reduction of *o*-nitrobenzylacetoacetic esters yields quinolines, not the dihydro compounds (112).

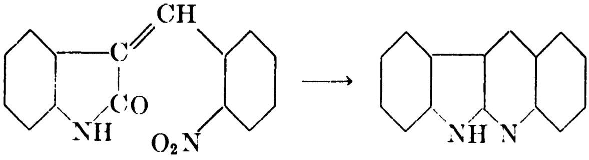
The *o*-nitrobenzoylketones of the type V on reduction yield substituted



V VI

2-phenyl-4-hydroxyquinolines (VI) (R = H, CN, or $COOC_2H_5$) (88).

The reduction of *o*-nitrobenzalisatin (VII) is of interest in that it leads to



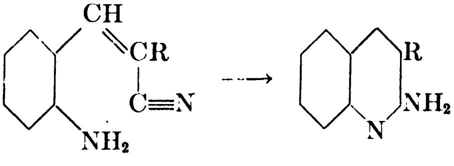
VII
o-Nitrobenzalisatin

VIII
peri-Quindoline

peri-quindoline (VIII), an example of a condensed compound containing both the indole and the quinoline nuclei.

The lone example of the addition of an *o*-amino group to a carbon-carbon double bond to yield a quinoline is recorded by Fischer and Kuzel (79). Reduction of *o*-nitrocinnamylideneacetone yielded 2-acetonylquinoline.

Strictly analogous is the addition of the *o*-amino group to the carbon-nitrogen triple bond in compounds of the formula IX, to yield 3-substituted 2-aminoquinolines of formula X.

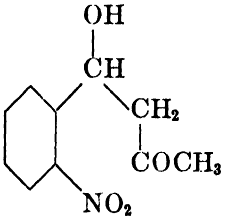


IX X

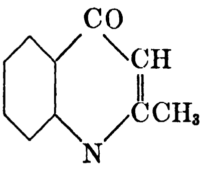
Compounds of formula IX are obtained by condensing *o*-acetylaminobenzaldehyde with arylacetonitriles and may yield the aminoacetylquinoline directly or on treatment with alkali (184). Acetonitrile does not condense with *o*-acetylaminobenzaldehyde, but its condensation product with *o*-nitrobenzaldehyde may be reduced to *o*-aminocinnamic nitrile, which on treatment with alkali yields 2-aminoquinoline.

Reduction of *o*-nitrobenzoylcyanoacetic ester with hydriodic acid and phosphorus yields 2-amino-4-hydroxyquinoline. The carbethoxyl group is eliminated under these conditions and 2,4-dihydroxy-3-cyanoquinoline is obtained (87).

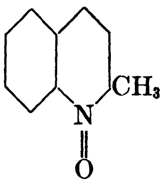
The condensation of *o*-nitrobenzaldehyde with acetone yields a representative of the so-called lactic methyl ketones (XI). This substance on gentle reduction gave a compound which Heller and Sourlis (102) regarded as being 4-keto-dihydroquinaldine (XII). It was shown, however, by Meisenheimer and



XI



XII



XIII

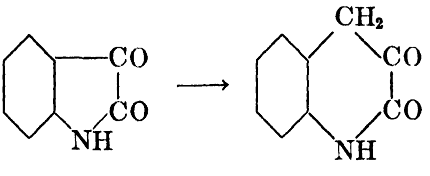
Stotz (157) to be identical with quinaldine *N*-oxide (XIII). The latter, of course, is readily obtainable by the oxidation of quinaldine with perbenzoic acid.

Miscellaneous syntheses

Under this heading it is proposed to group a number of syntheses which have resulted in compounds known to be, or regarded as being, quinolines. In a number of cases the mechanisms are obscure and frequently the reactions lack generality. In many cases the starting materials are difficultly accessible or the yields are poor.

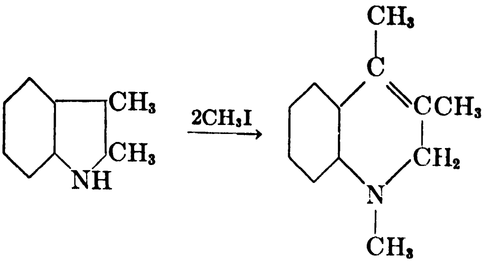
Dziewonski and Mayer (60) obtained 2-phenyl-3-methyl-4-aminoquinoline by heating a mixture of *sym*-diphenylthiourea and propiophenone to 250–260°C. Although a mechanism is postulated, it lacks conviction.

Heller (101) obtained 2,3-dihydroxyquinoline by the reaction of isatin in ether with diazomethane. This reaction finds an analogy in the ring enlargement of cyclic ketones by the same reagent.



An interesting example of a Beckmann rearrangement leading to a quinoline derivative is the preparation of hydrocarbostyril from the oxime of α-hydrindone (122). As is to be anticipated, no isoquinoline derivative is formed.

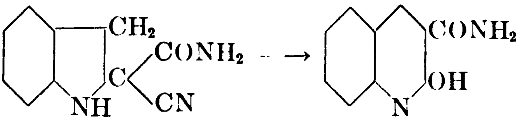
Fischer and Steche (80) have shown that the treatment of indoles with alkyl iodides at 100°C. yields dihydroquinolines carrying an alkyl group on the nitrogen atom. The ring is enlarged between the nitrogen atom and what is to become the β-carbon atom. With ethyl iodide, therefore, the resulting quinoline is a derivative of 1-ethyl-2-methyldihydroquinoline. The yields are good but the mechanism is obscure.



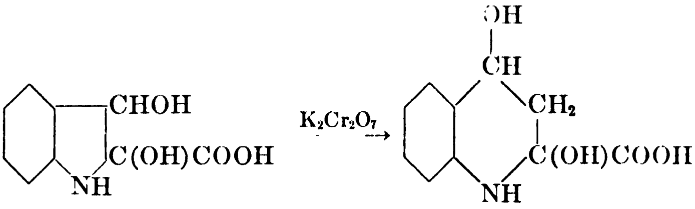
A similar transformation of an indole into a quinoline derivative was observed by Magnanini (150, 151). When 2-methylindole is heated with chloroform and sodium ethylate, it is converted into 2-methyl-3-chloroquinoline. Under the same conditions 3-methylindole yields 3-chloro-4-methylquinoline (69). The entering carbon atom takes up the β-position in the resulting quinoline, and the reaction appears to be quite general (121). Ellinger (68) has suggested a mechanism which, however, is not convincing.

Quinoline is obtained in small yield from either 1- or 2-methylindole when each is passed through a tube heated to dull redness (174).

Finally, mention may be made of two transformations of dihydroindoles into quinoline derivatives which were discovered by Heller and Wunderlich (103) and by Heller (100). In the first example nitrous fumes effected the change,



and the second example was written thus:

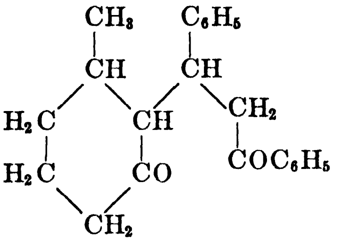


The source of the added carbon atom was not adequately explained, although the dihydroxy acid on treatment with acetic anhydride yielded quinaldinic acid.

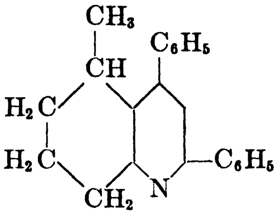
Other syntheses

There remain to be described a number of reactions which yield hydroquinolines from compounds in which the nitrogen is not already joined to the benzene nucleus. The resulting products are usually hydrogenated in the benzene nucleus.

Stobbe condensed β-methylcyclohexanone with benzalacetophenone, thus obtaining a 1,5-diketone (214, 215), which on treatment with hydroxylamine yielded II. The reaction is obviously a simple variation of the pyridine synthesis of Knoevenagel (125, 130). The position of the methyl group is in doubt.

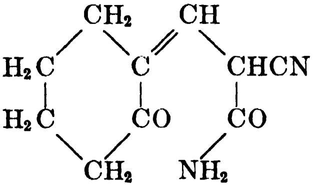


I

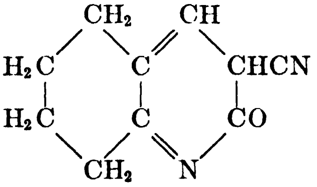


II

The condensation of hydroxymethylenecyclohexanone, i.e., formylcyclohexanone, with cyanoacetamide yields a substance formulated as III, which by further loss of water leads to the quinoline (IV) (204).

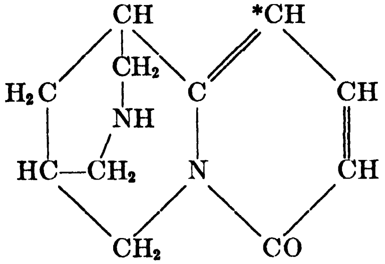


III

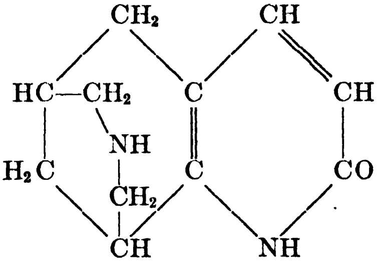


IV

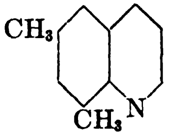
Knoevenagel and Fries (127, 128) condensed ethyl malonate with ethyl β-aminocrotonate and obtained a compound which was regarded as being 2-methyl-3-carbethoxy-4,5,7-trihydroxyquinoline.



V
Cytisine



VI



VII

Quinoline may be obtained from acridines under certain conditions. Acridine with hydrogen when passed over reduced nickel at 250–270°C. yielded a small amount of 2,3-dimethylquinoline (169). Oxidation of certain acridine derivatives leads to quinolinecarboxylic acids (107, 127).

The formation of 6,8-dimethylquinoline (VII) from cytisine (V) by heating with zinc dust (72) or with phosphorus and hydrogen iodide (83) is a rather remarkable example of a rearrangement which obscured the true constitution of the alkaloid for many years. Owing to the researches of Ing (110) and of Späth and coworkers (207, 208, 209), the true formula is now known. If it be assumed that the transformation to a quinoline derivative first involves the severance of the bond from tertiary nitrogen to CH_2 and a new ring is formed at the position marked with an asterisk, a substance of formula VI would result. It is obvious that the latter could readily lose the necessary elements to yield 6,8-dimethylquinoline. Further support to such a mechanism is given by the fact that cytisoline, the main product of the hydrogen iodide-phosphorus reaction, is the 2-hydroxy derivative of VII.

II. REACTIONS OF QUINOLINE

In the course of the very extensive work on quinoline and its derivatives, a large number of reactions of these compounds has been observed. In many cases, of course, the reactions are those met with in general organic chemistry and call for no special mention. Such reactions, as the esterification of acids or the hydrolysis of esters, in general follow the anticipated courses and will not be discussed further.

There are, however, a great many observations which could not have been predicted with certainty. They are in some measure peculiar to the chemistry of quinoline and call for some discussion. In the space available it is not possible to give a detailed account of even all salient observations. An attempt will be made to select typical reactions which admit of certain generalizations and to draw attention to some cases which seem more or less unique. The references quoted are not claimed to be complete. It is hoped, however, that the citations given will be adequate to trace any particular subject. For this reason it has been deemed desirable in many cases to give only the more recent references.

In the following pages there will be discussed first those reactions of quinoline and derivatives which do not specifically involve substituents. The next section will be devoted to reactions which involve the substituents.

A. Reactions not involving substituents

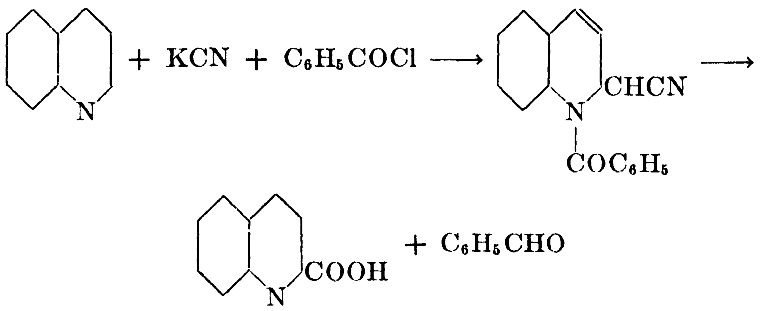
One of the earliest known reactions of quinoline is its reduction to tetrahydroquinoline by means of zinc and hydrochloric acid, and this reduction can be carried out with most alkyl and aryl derivatives. A variety of reducing agents may be used,—tin and hydrochloric acid, sodium amalgam (139), sodium and alcohol (226),—and in these cases only the pyridine nucleus is reduced.

Catalytic reduction of quinoline hydrochloride in ethanol in the presence of platinum oxide yields a mixture of *cis*- and *trans*-decahydroquinolines, whereas reduction of the base in glacial acetic acid yielded only the *trans*-modification (168). Reduction in the vapor state over nickel yields a mixture of products.

Good yields of tetrahydroquinoline were obtained by catalytic reduction in the presence of osmium and cerium dioxide (192). The tetrahydroquinolines are also obtainable by reducing the carbostyrils with sodium in absolute alcohol (73, 134). The nitroso derivative of tetrahydroquinoline on reduction yields 1-aminotetrahydroquinoline (108).

Oxidation of quinoline yields pyridine-2,3-dicarboxylic acid, and the preparation of this acid in good yield has been studied by Stix and Bulgatsch (213). Hydrogen peroxide is the reagent recommended. The oxidation is carried out in the presence of copper sulfate and the sparingly soluble copper salt separates as oxidation proceeds. The absence of iron is important.

Reissert (186) treated quinoline with benzoyl chloride in the presence of aqueous potassium cyanide. He obtained 1-benzoyl-2-cyano-1,2-dihydroquinoline, which on hydrolysis with hydrochloric acid yielded benzaldehyde and quinaldinic acid. The reaction has been extended by Sugasawa and Tsuda (217) to a number of substituted benzoyl chlorides with the object of preparing the corresponding aldehydes. In a number of cases the yields were good, but the reaction failed with phenylacetyl chloride and with heptoyl chloride.



Perbenzoic acid in benzene solution reacts readily with quinoline and its alkyl derivatives to yield the *N*-oxides (156). Mild reducing agents reconvert the latter to the original quinolines. Quinoline *N*-oxide reacts with sulfuryl chloride to give 4-chloroquinoline and a small amount of 2-chloroquinoline (21). With benzoyl chloride the *N*-oxide yields carbostyril, while the added presence of aqueous potassium cyanide results in the formation of 2-cyanoquinoline (106).

The nitration of quinoline has led to contradictory results, presumably because the conditions of the nitration have a decided influence on the course of the reaction: Schorigin and Toptschiew (194) obtained the 7-nitro- or the 5,7-dinitroquinoline, depending upon the temperature, when nitrogen peroxide was the nitrating agent. Bacharach, Haut, and Caroline (2) showed that nitration with fuming or with concentrated nitric acid and sulfuric acid yielded only the 5- and 8-nitroquinolines. However, a mixture of acetic anhydride, lithium nitrate, and copper nitrate yields exclusively the 7-nitro compound.

Doebner and v. Miller (55) reported that quinaldine on nitration in sulfuric acid yielded the 7- and the 8-nitro derivatives. Decker and Remfry (49) and Hammick (98) showed, however, that only the 5- and the 8-nitroquinaldines are obtained. The nitration of lepidine yields only the 8-nitro derivative (189), presumably because of the steric effect of the 4-methyl group on the 5-position.

The action of halogens leads to mixtures. Bromination has been studied under a variety of experimental conditions. In carbon disulfide a tetrabromoquinoline is formed, whereas bromine reacts with quinoline in the absence of solvent to yield a tetrabromide. An aqueous solution of a quinoline salt reacts with bromine at an elevated temperature to yield a mixture from which 3-bromoquinoline can be isolated in reasonable yield (38). The vapor-phase bromination has been investigated by Jansen and Wibaut (113). When the vapors were separately preheated and passed over pumice at 300°C., a 25 per cent yield of 3-bromoquinoline was obtained. At 450°C. a 24 per cent yield of 2-bromoquinoline was obtained, together with only a trace of the 3-bromo compound.

Quinoline and its derivatives readily react with alkyl halides, yielding *N*-alkylquinolinium halides (202). The latter are readily reduced (for example, by means of zinc and hydrochloric acid) to *N*-alkyltetrahydroquinolines (82), and this is certainly one of the best methods for obtaining such substances.

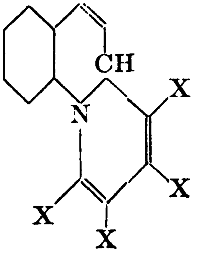
The alkylquinolinium hydroxides are strong water-soluble bases which on oxidation with potassium ferricyanide yield the *N*-alkyl-α-quinolones (48, 76). The *N*-alkylquinolinium iodides (or nitrates) in combination with the corresponding quinaldinium and lepidinium compounds and their derivatives yield, on treatment with sodium methylate, the so-called cyanine dyes, which have found extensive use in photography as photosensitizing agents. The study of these complex compounds and their application is a very specialized one and can be treated adequately only in a separate review.

Barium amide in liquid ammonia reacts with quinoline to yield 2-aminoquinoline with the elimination of hydrogen. Other amides give smaller yields and alkali amides give only resinous products (9).

Aryl and alkyl lithium compounds react with quinolines to yield largely 2-alkyl or 2-aryl-quinolines, the 1,2-dihydro compounds being obtainable as intermediates. Quinaldine yields a lithium derivative, which on treatment with alkyl halides is converted to alkylquinaldines, the alkyl group entering the side chains (231).

Aryl and alkyl Grignard reagents react with quinoline to yield insoluble addition compounds, which on heating to 150–160°C. rearrange to 2-aryl- and 2-alkyl-quinolines, respectively (10).

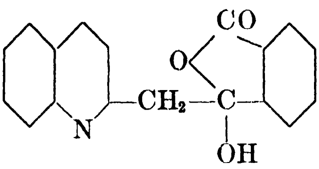
The Diels–Alder reaction with quinoline and quinaldine has been studied in detail (50). Two molecules of dimethyl acetylenedicarboxylate form a labile addition compound with quinoline, which on heating rearranges to the stable ester (I; X = $COOCH_3$).



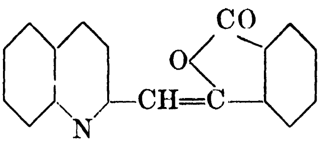
B. Reactions involving substituents

The simple alkyl derivatives of quinoline on oxidation frequently give rise to the acids carrying a carboxyl in place of the alkyl group. The methyl group in quinaldine or in lepidine but not that in 3-methylquinoline displays a special reactivity. The condensation of quinaldine (224) and of lepidine (224) with benzaldehyde and other aldehydes in the presence of zinc chloride leads to the formation of 2- and 4-styrylquinolines, respectively. Substituents frequently interfere with such reactions. Stark (212) failed to obtain condensation products with 3-aminoquinaldine or its acetyl derivative. Wislicenus and Kleisinger (229) condensed quinaldine and lepidine with ethyl oxalate by means of potassium ethylate. The compounds, $C_9H_6NCH_2COCOOC_2H_5$, were obtained in good yield. With chloral the products have the formula, $C_9H_6NCH_2CH(OH)CCl_3$, and these compounds can be hydrolyzed to β-quinolylacrylic acids with potassium carbonate (66).

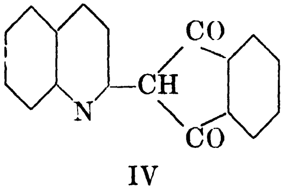
Eibner and Lange (64) and Eibner and Hofmann (63) made an extensive investigation of the reaction of quinaldine with phthalic anhydride. On gently heating a mixture of these substances, there is first formed a compound (II) which when further heated loses water and yields quinoisophthalone (III). This compound is moderately stable but on heating to a still higher temperature suffers a rearrangement to yield quinophthalone (IV). This reaction is of diagnostic value in identifying quinaldine and its derivatives and has been so used by Bailey and his coworkers in their study of the quinoline bases from petroleum distillates (143). The quinophthalones are sparingly soluble crystalline compounds of high melting point.



II



III
Quinoisophthalone



IV
Quinophthalone

Quinaldine on bromination yields only the ω-tribromoquinaldine and this on hydrolysis with dilute sulfuric acid yields quinaldinic acid (97). Selective bromination to a mono- or a di-bromo derivative could not be effected. However, reduction of the trichloroquinaldine with stannous chloride could be controlled to yield the dichloro or the monochloro derivatives. Treatment of these compounds with silver nitrate in ethanol or acetone gave quantitative yields of quinaldinic aldehyde and 2-quinolylcarbinol, respectively (98).

Hydroxyquinolines carrying the hydroxyl in the benzene ring behave like ordinary phenols. When the hydroxyl is in the pyridine nucleus it undergoes a number of reactions not common to a phenolic hydroxyl, although these compounds are soluble in strong alkalis. Heating with zinc dust in an atmosphere of hydrogen yields the corresponding quinoline (73, 131). The hydroxyl in position 2 or 4 is readily replaceable by chlorine when the compounds are treated with a mixture of phosphorus pentachloride and phosphorus oxychloride. These chloro compounds can be reduced to quinolines by heating with red phosphorus and hydrogen iodide (131). When 2-chloroquinoline is treated with potassium methylate, 2-methoxyquinoline is obtained and this when heated to a high temperature rearranges to yield *N*-methyl-2-keto-1,2-dihydroquinoline, which is also obtained by treating carbostyril with methyl iodide and alkali. The latter with phosphorus pentachloride in dichlorobenzene is easily reconverted to 2-chloroquinoline (77). The 2-hydroxyquinolines (i.e., the carbostyrils) are very weak bases, and the salts are almost completely hydrolyzed even in moderately strong acid solutions. The other hydroxyquinolines are in general as strong bases as the corresponding quinolines, so that in reactions where a mixture of the 2- and 4-hydroxyquinolines may be formed they are easily separable because of the difference in their solubilities in acid (33).

When 3-hydroxyquinoline is heated under pressure with ammonia, it yields 3-aminoquinoline (58). The Reimer-Tiemann reaction, that is, treatment with chloroform and alkali, with 4-hydroxyquinoline yields 4-hydroxy-3-aldehydoquinoline (20).

The aminoquinolines carrying the substituent in the hetero ring do not yield diazo compounds. When 4-aminoquinoline is treated with sodium nitrite in hydrochloric acid solution, it yields 4-chloroquinoline (227). 2-Phenyl-3-hydroxyquinoline can be prepared from the corresponding amino compound by treatment with nitrous acid (6).

In addition to the methods already described, the py-amino derivatives may be obtained by reducing the nitro compounds with tin and hydrochloric acid (43).

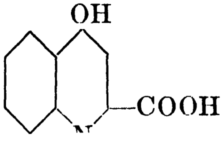
The 2- and 3-bromo derivatives of quinoline, when heated with cuprous cyanide, yield the corresponding nitriles and when heated with ammonia in the presence of copper powder yield the corresponding amines (114).

III. NATURAL OCCURRENCE OF QUINOLINES

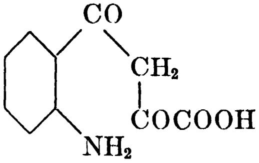
The most important occurrence of quinoline derivatives in plants is that of the cinchona and angostura alkaloids. The alkaloid echinopsine (95), present in *Echinops ritro*, was shown by Späth and Kolbe (210) to be identical with synthetic 1-methyl-4-quinolone. It was readily prepared by heating 4-hydroxyquinoline with methyl iodide in the presence of sodium methylate.

Suzuki and coworkers (218) isolated from rice bran several acids, one of which is regarded as a dihydroxyquinoline-5-carboxylic acid (193).

In animals the only quinoline derivative of importance is kynurenic acid (I). It occurs normally in the urine of dogs but not in that of cats. It is a product of the catabolism of tryptophan, the ultimate intermediate being *o*-aminobenzoylpyruvic acid



I
Kynurenic acid



II
o-Aminobenzoylpyruvic acid

The presence of quinoline or isoquinoline bases in petroleum distillates was demonstrated by Maberry and Wesson (148). Later, Bailey and coworkers in a series of researches isolated and identified a number of quinolines, among which may be mentioned the following: 2,3,8-trimethylquinoline (182); quinoline and quinaldine (28); 2,3,4,8-tetramethylquinoline and 2,3-dimethyl-8-propylquinoline (1); 2,4,8-trimethylquinoline and 2,4-, 2,3-, and 2,8-dimethylquinolines (17).

Ganguli and Guha (91) record the isolation of 5,8-dimethylquinoline. It was isolated as the picrate (m.p. 198°C.). The writer has prepared the same picrate and has found that it melts at 186°C.

REFERENCES

(1) Axe, W. N., and Bailey, J. R.: J. Am. Chem. Soc. **60**, 3028 (1938).
(2) Bacharach, G., Haut, A. H., and Caroline, L.: Rec. trav. chim. **52**, 413 (1933).
(3) Baeyer, A.: Ber. **12**, 1320 (1879).
(4) Baeyer, A., and Bloem, F.: Ber. **15**, 2147 (1882).
(5) Bamberger, E., and Kitschelt, M.: Ber. **27**, 3421 (1894).
(6) Bargellini, G., and Berlingozzi, S.: Gazz. chim. ital. **53**, 3 (1923).
(7) Barnett, E. de B.: Chem. News **121**, 205 (1920).
(8) Berend, L., and Thomas, E.: Ber. **25**, 2548 (1892).
(9) Bergstrom, F. W.: J. Am. Chem. Soc. **56**, 1748 (1934).
(10) Bergstrom, F. W., and McAllister, S. H.: J. Am. Chem. Soc. **52**, 2845 (1930).
(11) Bernthsen, A.: Ann. **224**, 1 (1884).
(12) Besthorn, E., Banzhof, E., and Jaeglé, G.: Ber. **27**, 3035 (1894).
(13) Besthorn, E., and Fischer, O.: Ber. **16**, 68 (1883).
(14) Besthorn, E., and Garben, E.: Ber. **33**, 3439, 3448 (1900).
(15) Besthorn, E., and Jaeglé, G.: Ber. **27**, 907, 3035 (1894).
(16) Beyer, C.: J. prakt. Chem. **33**, 393 (1886).
(17) Biggs, B. S., and Bailey, J. R.: J. Am. Chem. Soc. **55**, 4141 (1933).
(18) Bischler, A., and Burkart, E.: Ber. **26**, 1352 (1893).
(19) Blaise, E. E., and Maire, M.: Compt. rend. **144**, 93 (1907).

(20) Bobrański, B.: Ber. **69**, 1113 (1936).
(21) Bobrański, B.: Ber. **71**, 578 (1938).
(22) Böttinger, C.: Ann. **188**, 336 (1876).
(23) Braun, J. v., and Gruber, H.: Ber. **55**, 1710 (1922).
(24) Braunholz, W. T. K.: J. Chem. Soc. **121**, 169 (1922); J. Am. Chem. Soc. **44**, 2967 (1922).
(25) Borsche, W.: Ber. **41**, 3884 (1908).
(26) Borsche, W.: Ber. **42**, 4072 (1909).
(27) Borsche, W., and Jacobs, W.: Ber. **47**, 354 (1914).
(28) Bratton, A. C., and Bailey, J. R.: J. Am. Chem. Soc. **59**, 175 (1937).
(29) Busch, A., and Koenigs, W.: Ber. **24**, 3962 (1891).
(30) Camps, R.: Arch. Pharm. **237**, 659 (1899).
(31) Camps, R.: Ber. **32**, 3228 (1899).
(32) Camps, R.: Arch. Pharm. **239**, 591 (1901).
(33) Camps, R.: Arch. Pharm. **240**, 135 (1902).
(34) Chiozza, L.: Ann. **83**, 117 (1852).
(35) Ciusa, R.: Gazz. chim. ital. **52**, II, 43 (1922).
(36) Ciusa, R., and Zerbini, G.: Gazz. chim. ital. **50**, II, 317 (1920).
(37) Clarke, H. T., and Davis, A. W.: *Organic Syntheses*, Vol. II, p. 79. John Wiley and Sons, Inc., New York (1922).
(38) Claus, A., and Collischonn, F.: Ber. **19**, 2763 (1886).
(39) Clemo, G. R., and Perkin, W. H.: J. Chem. Soc. **125**, 1608 (1924).
(40) Clemo, G. R., and Perkin, W. H.: J. Chem. Soc. **127**, 2297 (1925).
(41) Cohn, B. E., and Gustavson, R. G.: J. Am. Chem. Soc. **50**, 2709 (1928).
(42) Cohn, E. W.: J. Am. Chem. Soc. **52**, 3685 (1930).
(43) Colonna, M.: Gazz. chim. ital. **67**, 46 (1937).
(44) Combes, A.: Compt. rend. **106**, 142 (1888).
(45) Combes, A.: Bull. soc. chim. [4] **49**, 89 (1888).
(46) Conrad, M., and Limpach, L.: Ber. **20**, 944, 948 (1887).
(47) Darzens, G., Delaby, R., and Hiron, J.: Bull. soc. chim. **47**, 227 (1930).
(48) Decker, H.: Ber. **25**, 443 (1892).
(49) Decker, H., and Remfry, P.: Ber. **38**, 2773 (1905).
(50) Diels, O., Alder, K., and coworkers: Ann. **510**, 87–128 (1934).
(51) Doebner, O.: Ber. **20**, 277 (1887).
(52) Doebner, O., and Fettback, H.: Ann. **281**, 1 (1894).
(53) Doebner, O., and Miller, W. v.: Ber. **14**, 2812 (1881).
(54) Doebner, O., and Miller, W. v.: Ber. **16**, 1664 (1883).
(55) Doebner, O., and Miller, W. v.: Ber. **17**, 1698 (1884).
(56) Drewsen, V. B.: Ber. **16**, 1953 (1883).
(57) D.R.P. 102,894 (1898).
(58) D.R.P. 611,691 (1935).
(59) D.R.P. 347,375 (1920).
(60) Dziewonski, K., and Mayer, J.: Roczniki Chem. **13**, 370 (1933).
(61) Edwards, M. G., Garrod, R. E., and Jones, H. O.: J. Chem. Soc. **101**, 1376 (1912).
(62) Eibner, A.: Ann. **318**, 58 (1901).
(63) Eibner, A., and Hofmann, K.: Ber. **37**, 3018 (1904).
(64) Eibner, A., and Lange, O.: Ann. **315**, 303 (1901).
(65) Eichengrün, A., and Einhorn, A.: Ber. **23**, 1489 (1890).
(66) Einhorn, A.: Ber. **19**, 904 (1886).
(67) Eliasberg, J., and Friedlaender, P.: Ber. **25**, 1752 (1892).
(68) Ellinger, A.: Ber. **39**, 2515 (1906).
(69) Ellinger, A., and Flamand, C.: Ber. **39**, 4388 (1906).
(70) Engelhard, C.: J. prakt. Chem. **57**, 467 (1898).
(71) Engler, C., and Riehm, P.: Ber. **18**, 2245 (1885).

(72) Ewins, A. J.: J. Chem. Soc. **103**, 97 (1913).
(73) Ewins, A. J., and King, H.: J. Chem. Soc. **103**, 104 (1913).
(74) Fichter, F., and Boehringer, R.: Ber. **39**, 3932 (1906).
(75) Fichter, F., and Rohner, F.: Ber. **43**, 3489 (1910).
(76) Fischer, O., and Chur, M.: J. prakt. Chem. **93**, 363 (1916).
(77) Fischer, O., and Guthmann, H.: J. prakt. Chem. **93**, 378 (1916).
(78) Fischer, E., and Kuzel, H.: Ann. **221**, 261 (1883).
(79) Fischer, E., and Kuzel, H.: Ber. **16**, 163 (1883).
(80) Fischer, E., and Steche, A.: Ann. **242**, 348 (1887).
(81) Foulds, R. P., and Robinson, R.: J. Chem. Soc. **105**, 1963 (1914).
(82) Freund, M.: Ber. **37**, 22 (1904).
(83) Freund, M.: Ber. **39**, 814 (1906).
(84) Friedlaender, P.: Ber. **15**, 2572 (1882).
(85) Friedlaender, P.: Ber. **47**, 3369 (1914).
(86) Froelicher, V., and Cohen, J. B.: J. Chem. Soc. **119**, 1431 (1921).
(87) Gabriel, S.: Ber. **51**, 1500 (1918).
(88) Gabriel, S., and Gerhard, W.: Ber. **54**, 1613 (1921).
(89) Gabriel, S., and Löwenberg, B.: Ber. **51**, 1493 (1918).
(90) Gabriel, S., and Thieme, A.: Ber. **52**, 1079 (1919).
(91) Ganguli, S. K., and Guha, P. C.: J. Indian Chem. Soc. **11**, 197 (1934).
(92) Garrod, R. E., Jones, H. D., and Evans, P. E.: J. Chem. Soc. **101**, 1389 (1912).
(93) Gattermann, L.: Ber. **27**, 1927 (1894).
(94) Graebe, C.: Ann. **201**, 333 (1880).
(95) Greshoff, M.: Rec. trav. chim. **19**, 360 (1900).
(96) Halberkann, J.: Ber. **54**, 3090 (1921).
(97) Hammick, D. L.: J. Chem. Soc. **123**, 2882 (1923).
(98) Hammick, D. L.: J. Chem. Soc. **1926**, 1302.
(99) Heller, G.: Ber. **43**, 1923 (1910).
(100) Heller, G.: Ber. **51**, 424 (1918).
(101) Heller, G.: Ber. **52**, 741 (1919).
(102) Heller, G., and Sourlis, A.: Ber. **41**, 2692 (1908).
(103) Heller, G., and Wunderlich, P.: Ber. **47**, 1617 (1914).
(104) Heller, G., and Wunderlich, P.: Ber. **47**, 2889 (1914).
(105) Henry, T. A.: *The Plant Alkaloids.* J. and A. Churchill, Ltd., London (1939).
(106) Henze, M.: Ber. **69**, 1566 (1936).
(107) Hepner, H.: Monatsh. **27**, 1045 (1906).
(108) Hoffmann, L., and Koenigs, W.: Ber. **16**, 727 (1883).
(109) Hollins, C.: *Synthesis of Nitrogen Ring Compounds.* Ernest Benn, Ltd., London (1924).
(110) Ing, H. R.: J. Chem. Soc. **1931**, 2195.
(111) Jackson, O. R.: Ber. **14**, 889 (1881).
(112) Jaenisch, A.: Ber. **56**, 2448 (1923).
(113) Jansen, H. E., and Wibaut, J. P.: Rec. trav. chim. **56**, 699 (1937).
(114) Jansen, H. E., and Wibaut, J. P.: Rec. trav. chim. **56**, 709 (1937).
(115) Johnson, J. R., and Adams, R.: J. Am. Chem. Soc. **45**, 1307 (1923).
(116) Jones, H. O., and Evans, P. E.: J. Chem. Soc. **99**, 334 (1911).
(117) Just, F.: Ber. **18**, 2632 (1885).
(118) Just, F.: Ber. **19**, 1462 (1886).
(119) Just, F.: Ber. **19**, 1541 (1886).
(120) Kaufmann, A., and Hüssy, H.: Ber. **41**, 1735 (1908).
(121) Kermack, W. O., Perkin, W. H., and Robinson, R.: J. Chem. Soc. **121**, 1882 (1922).
(122) Kipping, F. S.: J. Chem. Soc. **65**, 480 (1894).
(123) Kliegl, A., and Schmalenbach, A.: Ber. **56**, 1517 (1923).
(124) Knoevenagel, E.: Ber. **56**, 2414 (1923).

(125) Knoevenagel, E.: Ann. **281**, 25 (1894).
(126) Knoevenagel, E., and Bähr, H.: Ber. **55**, 1912 (1922).
(127) Knoevenagel, E., and Brunswig, R.: Ber. **35**, 2172 (1902).
(128) Knoevenagel, E., and Fries, A.: Ber. **31**, 767 (1898).
(129) Knoevenagel, E., and Goos, O.: Ber. **55**, 1929 (1922).
(130) Knoevenagel, E., and Weissgerber, R.: Ber. **26**, 436 (1893).
(131) Knorr, L.: Ann. **236**, 69 (1886).
(132) Knorr, L.: Ann. **245**, 357 (1888).
(133) Knorr, L. and Antrick, O.: Ber. **17**, 2870 (1884).
(134) Knorr, L., and Klotz, C.: Ber. **19**, 3299 (1886).
(135) Knorr, L., and Taufkirch, H.: Ber. **25**, 768 (1892).
(136) Knueppel, C. A.: Ber. **29**, 703 (1896).
(137) Koenigs, W.: Ber. **12**, 453 (1879).
(138) Koenigs, W.: Ber. **13**, 911 (1880).
(139) Koenigs, W.: Ber. **14**, 98 (1881).
(140) Koenigs, W., and Meimberg, F.: Ber. **28**, 1038 (1895).
(141) Kulisch, V.: Monatsh. **15**, 276 (1894).
(142) Kulisch, V.: Monatsh. **16**, 351 (1895).
(143) Lake, G. R., and Bailey, J. R.: J. Am. Chem. Soc. **55**, 4141 (1933).
(144) Lake, G. R., and Bailey, J. R.: J. Am. Chem. Soc. **55**, 4143 (1933).
(145) Lellmann, E., and Alt, H.: Ann. **237**, 307 (1887).
(146) Lellmann, E., and Schleich, C.: Ber. **20**, 434 (1887).
(147) Lellmann, E., and Schmidt, O.: Ber. **20**, 3154 (1887).
(148) Maberry, C. F., and Wesson, L. G.: J. Am. Chem. Soc. **42**, 1014 (1920).
(149) Madelung, W.: Ber. **45**, 3521 (1912).
(150) Magnanini, G.: Ber. **20**, 2608 (1887).
(151) Magnanini, G.: Ber. **21**, 1940 (1888).
(152) Mann, F. G.: J. Chem. Soc. **121**, 2178 (1922).
(153) Marckwald, W., and Berndt, L.: Ann. **274**, 31 (1893).
(154) Marckwald, W., and Schmidt, C.: Ann. **274**, 367 (1893).
(155) Mayer, F., van Zütphen, L., and Philipps, H.: Ber. **60**, 858 (1927).
(156) Meisenheimer, J.: Ber. **59**, 1848 (1926).
(157) Meisenheimer, J., and Stotz, E.: Ber. **58**, 2334 (1925).
(158) Meyer, E. v.: J. prakt. Chem. **90**, 1 (1914).
(159) Meyer, H., and Beer, R.: Monatsh. **34**, 1173 (1913).
(160) Miller, W. v., and Kinkelin, F.: Ber. **19**, 525 (1886).
(161) Miller, W. v., and Kinkelin, F.: Ber. **20**, 1916 (1887).
(162) Miller, W. v., and Plöchl, J.: Ber. **29**, 1462 (1896).
(163) Mills, W. H., Harris, J. E. G., and Lambourne, H.: J. Chem. Soc. **119**, 1294 (1921).
(164) Neber, P. W.: Ber. **55**, 826 (1922).
(165) Niementowski, S., and Orzechowski, B.: Ber. **28**, 2809 (1895).
(166) Niementowski, S.: Ber. **27**, 1394 (1894).
(167) Nölting, E., and Steuer, O. R.: Ber. **43**, 3512 (1910).
(168) Overhoff, J., and Wibaut, J. P.: Rec. trav. chim. **50**, 957 (1931).
(169) Padoa, M., and Fabris, U.: R.A.L. [V] **16**, I, 921 (1907).
(170) Perkin, W. H., and Plant, S. G. P.: J. Chem. Soc. **123**, 676 (1923).
(171) Perrin, T. S., and Bailey, J. R.: J. Am. Chem. Soc. **55**, 4136 (1933).
(172) Pfitzinger, W.: J. prakt. Chem. **33**, 100 (1886).
(173) Pfitzinger, W.: J. prakt. Chem. **66**, 263 (1902).
(174) Pictet, A.: Ber. **38**, 1946 (1905).
(175) Pictet, A., and Barbier, H.: Bull. soc. chim. **13**, 26 (1895).
(176) Pictet, A., and Barbier, H.: Bull. soc. chim. **13**, 28 (1895).
(177) Pictet, A., and Bunzl, R.: Ber. **22**, 1847 (1889).
(178) Pictet, A., and Duparc, L.: Ber. **20**, 3415 (1887).

(179) Pictet, A., and Fert, J.: Ber. **23**, 1903 (1890).
(180) Pictet, A., and Misner, R. R.: Ber. **45**, 1800 (1912).
(181) Pinkus, G.: Ber. **25**, 2798 (1892).
(182) Poth, E. J., *et al.*: J. Am. Chem. Soc. **52**, 1239 (1930).
(183) Prud'homme, M.: Bull. soc. chim. [2] **28**, 62 (1877).
(184) Pschorr, R.: Ber. **31**, 1289 (1898).
(185) Reed, J. H.: J. prakt. Chem. **32**, 630 (1885).
(186) Reissert, A.: Ber. **38**, 1603 (1905).
(187) Rindfusz, R. E., and Harnack, V. L.: J. Am. Chem. Soc. **42**, 1720 (1920).
(188) Rist, E.: Ber. **23**, 3483 (1890).
(189) Roberts, E., and Turner, E. E.: J. Chem. Soc. **1927**, 1832.
(190) Rohde, G.: Ber. **20**, 1911 (1887).
(191) Rügheimer, L.: Ber. **17**, 736 (1884).
(192) Sadikov, V. S., and Mikhailov, A. K.: J. Chem. Soc. **1928**, 438.
(193) Sahashi, Y.: Biochem. Z. **159**, 221 (1925).
(194) Schorigin, P., and Toptschiew, A.: Ber. **69**, 1874 (1936).
(195) Schutzenberger, P.: Compt. rend. **85**, 147 (1877).
(196) Simon, L. J.: Compt. rend. **144**, 138 (1907).
(197) Simon, L. J.: Compt. rend. **146**, 1400 (1908).
(198) Simon, L. J.: Compt. rend. **147**, 125 (1908).
(199) Simon, L. J., and Conduché, A.: Compt. rend. **139**, 297 (1904).
(200) Simon, L. J., and Mauguin, C.: Compt. rend. **144**, 1275 (1907).
(201) Skraup, Z. H.: Ber. **13**, Ref. 2086 (1880); **15**, 897 (1882).
(202) Skraup, Z. H.: Monatsh. **2**, 139 (1881).
(203) Skraup, Z. H.: Ber. **15**, 893 (1882).
(204) Sen-Gupta, H. K.: J. Chem. Soc. **107**, 1347 (1915).
(205) Smirnoff, A. P.: Helv. Chim. Acta **4**, 802 (1921).
(206) Spallino, R., and Salimei, G.: Gazz. chim. ital. **42**, 607 (1912).
(207) Späth, E.: Monatsh. **40**, 15, 93 (1919).
(208) Späth, E., and Galinovsky, F.: Ber. **66**, 1338 (1933).
(209) Späth, E., and Galinovsky, F.: Ber. **71**, 721 (1938).
(210) Späth, E., and Kolbe, A.: Monatsh. **43**, 469 (1922).
(211) Stark, O.: Ber. **40**, 3425 (1907).
(212) Stark, O.: Ber. **46**, 2697 (1913).
(213) Stix, W., and Bulgatsch, S. A.: Ber. **65**, 11 (1932).
(214) Stobbe, H.: Ber. **35**, 3978 (1902).
(215) Stobbe, H.: J. prakt. Chem. **86**, 218 (1912).
(216) Stuart, C. M.: J. Chem. Soc. **47**, 155 (1885).
(217) Sugasawa, S., and Tsuda, T.: J. Pharm. Soc. Japan. **56**, 103 (1936).
(218) Suzuki, U., Shimamura, T., and Odake, S.: Biochem. Z. **43**, 89 (1912).
(219) Thielepape, E.: Ber. **55**, 127 (1922).
(220) Tiemann, F., and Oppermann, J.: Ber. **13**, 2070 (1880).
(221) Tortelli, M.: Gazz. chim. ital. **16**, 366 (1886).
(222) Tröger, J., and Koppen-Kastrop, P.: J. prakt. Chem. **104**, 335 (1922).
(223) Tröger, J., and Menzel, W.: J. prakt. Chem. **103**, 188 (1921).
(224) Wallach, O., and Wüsten, M.: Ber. **16**, 2007 (1883).
(225) Walther, R. v.: J. prakt. Chem. **67**, 504 (1903).
(226) Weidel, H., and Gläser, G.: Monatsh. **7**, 308 (1887).
(227) Wenzel, F.: Monatsh. **15**, 453 (1894).
(228) Wislicenus, W., and Elvert, H.: Ber. **42**, 1144 (1909).
(229) Wislicenus, W., and Kleisinger, E.: Ber. **42**, 1140 (1909).
(230) Wohnlich, E.: Arch. Pharm. **251**, 526 (1913).
(231) Ziegler, K., and Zeiser, H.: Ann. **485**, 174 (1931).

THE CHEMISTRY OF ISOQUINOLINES

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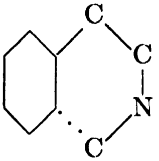
This review deals with (*a*) systematization of all reactions which lead to the synthesis of the isoquinoline nucleus, (*b*) the reactions of isoquinolines which are characteristic of the nucleus, and (*c*) reactions of substituted isoquinolines which are occasioned by the presence of the nucleus. The chemistry of the isoquinoline alkaloids is not specifically treated, except when it is included in one of the above categories.

As in the case of the quinolines, the syntheses of isoquinoline and its derivatives may be divided into a number of types, depending upon the point at which the hetero ring is closed. There are, therefore, five possible ways in which these compounds can be theoretically prepared, giving rise to five type syntheses: namely, ring closure between the nucleus and carbon atom 1 (Type I), between atoms 1 and 2 (Type II), between atoms 2 and 3 (Type III), between carbon atoms 3 and 4 (Type IV), and between carbon atom 4 and the nucleus (Type V). In each of the following formulas the dotted line indicates the point at which union is to be effected.



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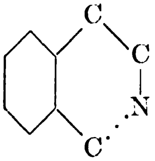

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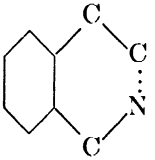

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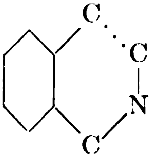

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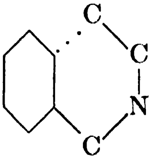


V

Mechanisms representative of all types are known, but in many cases the compounds obtained are keto derivatives of tetrahydroisoquinolines, some of which are convertible into isoquinolines or their tetrahydro derivatives only with difficulty. No attempt will be made in the following review to refer to all, or even most, of the many compounds which have been prepared (57). It is deemed sufficient to discuss the various synthetic procedures. It is hoped that the references cited will serve as a basis for a more thorough search of the literature on any particular phase of the subject.

Following the discussion of synthesis, there is a review of the reactions of isoquinolines. In this section those reactions which are common to organic compounds in general will not be treated.

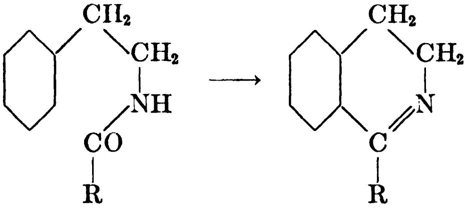
It is difficult to review the chemistry of isoquinolines without reference to alkaloids, since much of the chemistry of the former has been built up in attempts to synthesize alkaloids or their degradation products. Then, too, many reactions of the isoquinolines have been encountered in researches concerned with alkaloids. Nevertheless, Henry (56) has adequately reviewed the subject of the isoquinoline alkaloids, both from the synthetic and the degradative points of view, so that references to alkaloid chemistry will be greatly restricted.

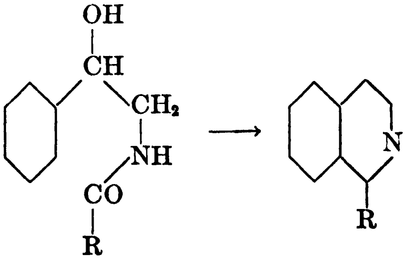
I. SYNTHESES OF ISOQUINOLINE AND ITS DERIVATIVES

Type I: Syntheses from β-arylethylamines

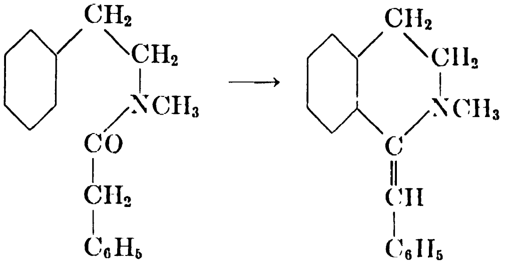
The reactions described as of Type I (i.e., ring closure between the nucleus and what is to become the 1-carbon atom) necessitate as a primary starting material a β-arylethylamine or some substance which during the synthesis will give rise to such an amine or a derivative. Consequently, most of the researches dealing with the synthesis of β-arylethylamines have a direct bearing on the synthesis of isoquinolines. In this connection it is therefore pertinent to mention that Schales (92) has reviewed the literature on β-arylethylamines. Mention may also be made of the researches of Kindler and coworkers (64, 65) on new and improved syntheses of these bases and of their β-hydroxy derivatives. Important too is the work on the arylacetic and β-arylpropionic acids, the former of which are used as the source of the 1-benzyl substituent in the synthetic benzylisoquinolines (66).

The synthesis of Bischler and Napieralski (10) was achieved by heating the acetyl or the benzoyl derivative of β-phenylethylamine with phosphorus pentoxide or with zinc chloride at about 200°C. The products were 1-alkyl- or 1-aryl-3,4-dihydroisoquinolines. The yields were only mediocre, but the later work of Pictet (81, 82) and of Decker (22, 23) and their coworkers effected material improvements so that the reaction became a practical one for the synthesis of isoquinoline alkaloids. The reaction is now generally carried out in boiling toluene or xylene in the presence of phosphorus pentoxide or phosphorus oxychloride, although aluminum chloride, ferric chloride, zinc chloride, and phosphorus pentachloride have been used. The benzylisoquinoline alkaloids,—laudanosine, laudanine, and papaverine; cotarnine and hydrastinine; salsoline, pectenine, and corypallin; and the anhalonium alkaloids,—have been synthesized by this reaction. When the β-aryl-β-hydroxyethylamines are used as the starting materials, an additional molecule of water is eliminated and isoquinolines are produced (79, 80).



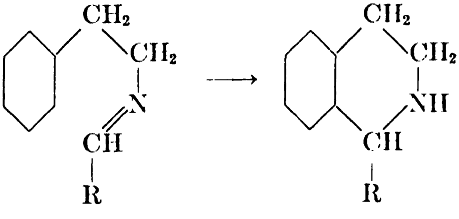


When R is phenyl, the double bond introduced as the result of ring closure can take only the position between carbon atom 1 and the nitrogen atom. When R is benzyl or a homologous radical, however, the double bond could be outside of the ring. This happens when the nitrogen is methylated. From phenylacetyl(β-phenylethyl)methylamine Hamilton and Robinson (53) obtained 1-benzal-*N*-methyltetrahydroisoquinoline.



The record of complete failures does not include many examples. Formyl-(β-phenylethyl)amine yields only a small amount of dihydroisoquinoline (23), the main product being a complex aminomalonamide (20).

An important variation of the Bischler-Napieralski reaction was introduced by Pictet and Spengler (84), when they condensed aldehydes with β-arylethylamines and effected ring closure by means of hot hydrochloric acid. Phenylalanine yielded a small amount of an isoquinolinecarboxylic acid, but tyrosine and tyramine gave better results. Decker and Becker (21) extended this work to the methylenedioxyarylamines and a number of aldehydes. The synthesis of harman from tryptophan (61, 77) is an example of a similar ring closure in which the carboxyl group is eliminated and the hydro-hetero ring is oxidized at the same time.



Of great biological interest is the condensation of β-arylethylamines with aldehydes and pyruvic acids under the so-called physiological conditions. Condensation is brought about at room temperature under controlled conditions of pH. Schöpf and Bayerle (94) condensed β-(3,4-dihydroxyphenyl)ethylamine hydrobromide with a slight excess of acetaldehyde. At 25°C. and pH 3 to 5 an 83 per cent yield of 1-methyl-6,7-dihydroxytetrahydroisoquinoline was obtained. Hahn and Schales (51, 52) succeeded in obtaining a 5 per cent yield of 3′,4′,6,7-bismethylenedioxytetrahydroprotopapaverine by condensing β-piperonylethylamine with homopiperonal under similar conditions. Such condensations under physiological conditions proceed easily only when the ring to which closure is effected is suitably substituted with free hydroxyl groups. It is argued that such are the conditions which prevail in plants and that methylation and methylenation are subsequently achieved.

Kaufmann and Dürst (63) have modified the synthesis with formaldehyde by condensing the arylamine first with chloromethylmethyl ether. The *N*-methoxymethyl derivative may be isolated as an intermediate and the ring ultimately closed by treatment with phosphorus pentoxide or with hot dilute hydrochloric acid.

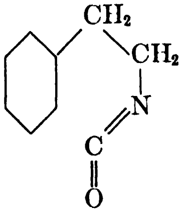
Type I: Other syntheses

There are a variety of reactions which yield β-arylethylamines as ultimate products. The intermediates are frequently obtainable as stable compounds and in some cases the disposition of reactive groups is such that ring closure to an isoquinoline is possible.

The Beckmann rearrangement of oximes leads to acyl or aroyl amines and, in the case of the oxime of cinnamaldehyde, the intermediate formylstyrylamine yields a small amount of isoquinoline (5, 44) when treated with phosphorus pentoxide. Zelinsky (102) had obtained an oily base from the oxime of styryl methyl ketone under similar conditions. This was shown by Goldschmidt (45) to be isoquinoline, not the expected 1-methylisoquinoline, which was only obtained at a later date by Burstin (14) from the same starting materials. Styryl phenyl ketone failed to yield an isoquinoline (46).

Much better yields of isoquinolines (in this case the 3,4-dihydro compounds) are obtainable from the oximes of saturated ketones. From the oxime of benzylacetone Pictet and Kay (82) obtained 1-methyl-3,4-dihydroisoquinoline in a yield of approximately 50 per cent. Sugasawa and Yoshikawa (98) condensed veratraldehyde with acetone, reduced the distryryl compound, and then prepared the oxime. The latter, on heating with phosphorus oxychloride in toluene, yielded a tetramethoxy-1-(β-phenylethyl)dihydroisoquinoline.

The Hofmann synthesis of amines produces as one of the intermediates an isocyanate (I), which might be expected to undergo ring closure with the formation of dihydroisocarbostyril (II). Weerman and Jongkees (100) failed to achieve this synthesis with the unsubstituted compound. In the Curtius synthesis of amines it is easier to isolate the intermediate isocyanate, and Mohunta and Ray (75) have succeeded in effecting the ring closure of the isocyanate (III) to corydaldine (IV) by means of phosphorus oxychloride. The reaction is an adaptation of the more facile one in which the azide of β-3-indolylpropionic acid,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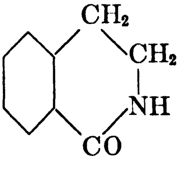
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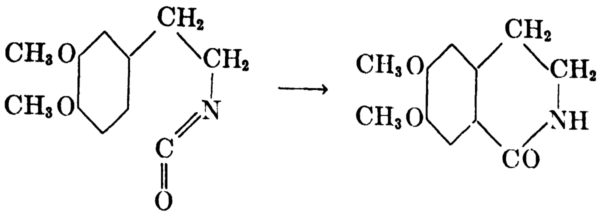
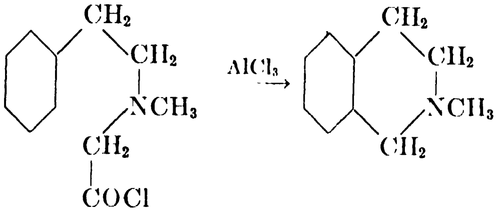


III IV
Corydaldine

on boiling in benzene in a stream of hydrogen chloride, yielded 3-keto-3,4,5,6-tetrahydro-4-carboline (72). The earlier synthesis of corydaldine by Späth and Dobrowsky (95) was achieved by treating the reaction product of ethyl chloroformate and β-veratrylethylamine with phosphorus pentoxide in xylene. It is closely related to the above.

Bruckner and Krámli (13) described a synthesis of 1-alkyl-3-methyl-6,7-methylenedioxyisoquinolines. The N-acyl-β-hydroxyarylethylamines were obtained from isosafrole by a series of reactions involving nitroso compounds.

v. Braun and Wirz (11) prepared a series of β-arylethylglycines with or without a methyl group on the nitrogen. The acid chlorides of these (V) on treatment with aluminum chloride yielded not the expected homoisocarbostyrils but the tetrahydroisoquinolines (VI) together with carbon monoxide. When the nitrogen was secondary, it was necessary to make the p-toluenesulfonyl derivative first.



V VI

Type II syntheses

The reactions classed as Type II which lead to the synthesis of isoquinoline derivatives are greatly limited in generality and in usefulness. The condensation of a β-arylethylamine containing a reactive ortho-substituent can yield a quinoline, but these compounds are in general accessible only with difficulty.

Bain, Perkin, and Robinson (2) utilized the hippuric acid condensation in converting o-carboxybenzaldehyde into 1-hydroxy-3-carboxyisoquinoline. The azlactone was hydrolyzed with 10 per cent caustic potash and the intermediate suffered ring closure during the course of the hydrolysis. Opianic acid gave the corresponding dimethoxy derivative. Dihydroisocarbostyril was prepared from N-benzoyl-β-(o-carboxyphenyl)ethylamine by Bamberger and Dieckmann (3). This particular reaction, however, can be of limited use only, because their ultimate intermediate was prepared from *N*-benzoyltetrahydroisoquinoline by oxidation with permanganate.

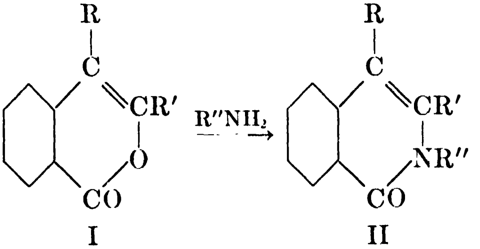
More complex syntheses have been reported by Schneider and Schroeter (93) and by Gabriel (38).

Homophthalimide is 1,3-diketo-1,2,3,4-tetrahydroisoquinoline. It is obtainable from homophthalic acid by treatment with ammonia and when heated with zinc dust (70), or successively with phosphorus oxychloride and hydriodic acid (36), it yields isoquinoline. Homophthalimide is also obtainable from *o*-cyanobenzyl cyanide on hydrolysis with sulfuric acid (37) and both syntheses, but particularly the former, are of wide applicability.

Closely related to the above syntheses is one in which isocarbostyril is obtained directly. The amide of *o*-cyanocinnamic acid on treatment with sodium hypochlorite in methanol yields first the carbomethoxyl derivative of *o*-cyanostyrylamine. When the latter is heated with hydrochloric acid, ammonia, methanol, and carbon dioxide are eliminated and isocarbostyril is formed (39).

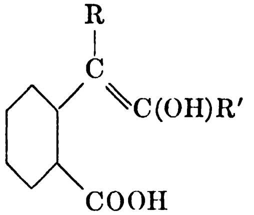
When the hydrochlorides of α,ω-diamino compounds in which the amino groups are separated by four or five carbon atoms are distilled, ammonia is eliminated and a pyrrolidine or a piperidine is formed. Helfer (55) distilled the dihydrochloride of β-(*o*-aminomethylphenyl)ethylamine and obtained tetrahydroisoquinoline in nearly 60 per cent yield. When homoxylylene dibromide is treated with aniline, it yields *N*-phenyltetrahydroisoquinoline. With dimethylamine a quaternary bromide is obtained (12).

Primary amines react with isocoumarins to give in most cases quantitative yields of the corresponding isoquinoline derivatives. Gabriel (32, 33, 34, 35) prepared the compound I (R = H; R′ = C_6H_5). This on treatment with ammonia yields 3-phenylisocarbostyril (II) (R = R″ = H; R′ = C_6H_5). The reaction has been extended by Heilmann (54) and by Bethmann (9). The isocoumarins are difficultly accessible compounds and a variety of syntheses have been elaborated. The reaction of benzoyl chloride with *o*-cyanobenzyl cyanide, whereby 3-phenyl-4-cyanoisocoumarin may be obtained (42), is not as suitable for analogous alkyl compounds because the yields are low (43). Ingenious, but applicable only to the synthesis of isocoumarin, is the oxidation of β-naphthoquinone with sodium hypochlorite. The isocoumarin and the intermediate carboxylic acid on treatment with ammonia yield isocarbostyril and isocarbostyril-3-carboxylic acid, respectively (4).

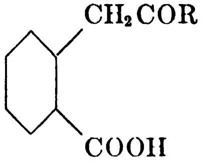


I II

The esters of isocoumaric acid (III) may be prepared by the Claisen condensation of the esters of homophthalic acid with aliphatic and aromatic esters. Treatment of these with ammonia yields isocarbostyrils (24). The *o*-phenacyl- and *o*-acetonyl-benzoic acids (IV) ($R = C_6H_5$ or CH_3) are the ketonic tautomers of isocoumaric acids, and as such condense with primary amines to yield isocarbostyrils (33, 48). The reaction has been extended to a number of hydroxy- and ethoxy-isocoumarins by Fritsch (30). The action of hydrazines on isocoumarins yields compounds which are either the *N*-aminoisocarbostyrils or seven-membered ring compounds containing two nitrogen atoms in the ring. On reduction with zinc and hydrochloric acid, they lose ammonia or arylamines and thus behave like substituted hydrazines. The products are isocarbostyrils in which the nitrogen atom is secondary (48, 71, 101).



III
Isocoumaric acid



IV

Type III syntheses

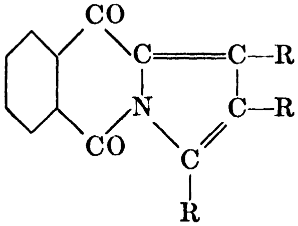
In the reactions of Type III, ring closure is brought about by a union between the nitrogen atom and what is to become carbon atom 3 in the ring. No unambiguous examples of such a synthesis are known. It is probable that many of the reactions described as of Type II properly belong here. The exact mechanism, however, is obscure and for the present purpose of no consequence.

Type IV syntheses

Ring closure between carbon atoms 3 and 4 to yield isoquinolines is restricted to a few examples, all of which involve the condensation of a nuclear carboxyl with an active hydrogen.

The first and typical example is one discovered by Gabriel and Colman (40, 41), in which ethyl phthalimidoacetate was treated with sodium ethylate. A modified Dieckmann reaction takes place, in which the phthalimido ring is first opened and then re-formed at the methylene carbon. The ultimate product is 3-carbethoxy-4-hydroxyisocarbostyril. The synthesis is limited to phthalimido derivatives containing a group which will undergo the Claisen condensation. Phthalimido derivatives of simple ketones (68), but not benzylphthalimide, yield isocarbostyrils. When the phthalyl derivatives of amino acids, other than that of glycine, are used, the carboxyl group is eliminated and the products are 3-alkyl(or aryl)-4-hydroxyisocarbostyrils (40, 99). In a few cases this reaction has been applied to compounds substituted in the phthalyl moiety (29, 69).

Closely related but distinctly novel is the modification described by Fischer and Krollpfeiffer (28). 2,3,4-Trisubstituted pyrroles condense with phthalic anhydride in the presence of acetic anhydride to give rise to compounds of formula I. It is probable that an *o*-carboxybenzoylpyrrole is formed as an intermediate.



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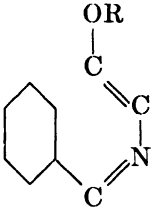
Tetraalkylpyrroles give the same product, one alkyl group being eliminated.

Type V syntheses

There is no isoquinoline synthesis as elegant and useful as the Skraup quinoline synthesis. Nevertheless some reactions classed as Type V (i.e., ring closure between carbon atom 4 and the nucleus) bear a close resemblance to some of the modifications of the Skraup synthesis. Staub (97), as the result of a comprehensive study, has defined the structural conditions necessary for the formation of isoquinoline derivatives. Ring closure can occur only in compounds of formula I or II, the conjugated double bonds being actually or potentially present. The terminal carbon carries an OH or OR group which is eliminated during the condensation. Benzalaminoacetal, on treatment with phosphorus oxychloride or sulfuric acid, gives isoquinoline in a yield of about 50 per cent (30, 85, 86). Here, the elimination of one OC_2H_5 group produces the required second double bond. In the synthesis from benzylaminoacetaldehyde (27) ring closure is effected by oleum, which oxidizes the benzyl to a benzal derivative, the enol of the resulting compound having formula II. Rügheimer and Schön (91), in their synthesis of 6,7-dimethoxyisoquinoline by a similar method, used arsenic pentoxide as the oxidizing agent. In Perkin and Robinson's (76) synthesis of the 7,8-dimethoxy derivative, ring closure was effected by 70 per cent sulfuric acid. The necessary acetal, with one actual double bond, was obtained by condensing aminoacetal with 2,3-dimethoxybenzaldehyde. The enolic form of benzoylaminoacetal has one actual and one potential double bond



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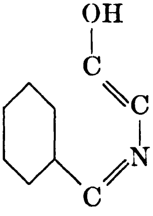
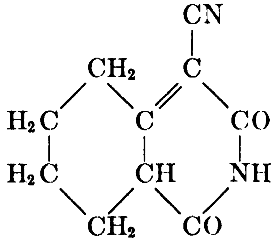


II

and yields isocarbostyril on treatment with sulfuric acid (30). When there is a *m*-hydroxyl or *m*-alkoxyl group in the benzene nucleus, ring closure yields the 7- rather than the 5-substituted isoquinolines (31).

Benzoylaminoacetic acid, when treated with an excess of phosphorus pentachloride, yields 1,4-dichloroisoquinoline, presumably by way of 4-hydroxyisocarbostyril (90). An analogous reaction using 3,4-dimethoxybenzalglycine failed to yield an isoquinoline (76).

Basu (6) and Basu and Banerjee (7) have adapted the Guareschi synthesis (50) of pyridines and its modification by Knoevenagel and Fries (67) to the synthesis of bz-hexahydroisoquinolines. In the first example, ethyl cyclohexanonecarboxylate was condensed with cyanoacetamide to yield compound III. In the second example, oxymethylenecyclohexanone was treated with ammonia and the resulting aminomethylene compound was condensed with ethyl acetoacetate in the presence of sodium. The resulting substance (IV) on hydrolysis yielded the corresponding acid, which was easily decarboxylated and converted into isoquinoline by distillation with zinc dust.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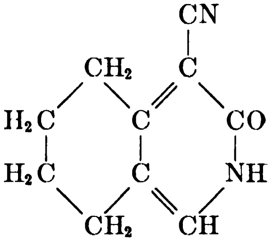
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Miscellaneous syntheses

Only two syntheses of isoquinoline are considered here and both depend upon pyrolysis. Graebe and Pictet (49) obtained a small yield of a base, by distilling *N*-methylisoindolinone with zinc dust, which was later identified as isoquinoline (78).

When benzalethylamine is passed through a hot tube, some isoquinoline can be isolated from the mixture of products (83).

II. REACTIONS OF ISOQUINOLINE AND ITS DERIVATIVES

The reactions of isoquinoline and its derivatives may be divided into those which are not dependent upon the reactivity of substituents and those which are. Reactions which are common to organic compounds in general and are not dependent upon the presence of the isoquinoline part of the molecule will not come under consideration in this review.

A. Reactions not involving substituents

The reduction of isoquinolines to the tetrahydro bases can be brought about by tin and hydrochloric acid (58) or catalytically in the presence of platinum oxide (88). When isoquinoline is oxidized in alkaline solution with permanganate, there is formed a mixture of pyridine-3,4-dicarboxylic acid, phthalic acid, and oxalic acid as well as ammonia and a mixture of unidentified compounds (58). When the oxidation is carried out in neutral solution with permanganate, the chief product is phthalimide (47). Oxidation with perbenzoic acid yields isoquinoline *N*-oxide (73).

When isoquinoline is treated with bromine, there is formed an addition product which when heated to 180–200°C. yields a py-bromoisoquinoline. The latter on oxidation with permanganate yields a bromopyridinedicarboxylic acid of uncertain constitution (26).

The nitration of isoquinoline in fuming sulfuric acid yields a nitro derivative which on oxidation with nitric acid or with permanganate yields 3-nitrophthalic acid (17). It is, therefore, a 5- or an 8-nitroisoquinoline or a mixture of these (1). Similarly, sulfonation yields a mixture of the α- and β-isoquinolinesulfonic acids which are separable by means of their barium salts. The α-form predominates when the reaction is carried out at 115°C., and the β-form at 250–260°C. (18, 19). When the barium salt of the α-sulfonic acid is heated in a current of hydrogen with potassium ferrocyanide, a small yield of a bz-cyanoisoquinoline is obtained. The cyano group of this compound is saponifiable only with difficulty (62).

When isoquinoline is treated with sodium amide in neutral solvents, it yields 1-aminoisoquinoline. The latter does not yield a diazo compound, but on treatment with nitrous acid is converted to isocarbostyril (15). The introduction of an alkyl group into the 1-position by means of the Grignard reaction was described by Bergstrom and McAllister (8). There is first formed an addition compound which suffers a rearrangement when heated to 150–160°C.

The reaction of benzoyl chloride with isoquinoline yields only an unstable addition compound from which isoquinoline can be easily regenerated. In the presence of added potassium cyanide, there is formed 1-cyano-*N*-benzoyl-1,2-dihydroisoquinoline. This on hydrolysis with hydrochloric acid yields isoquinoline-1-carboxylic acid and benzaldehyde (89). The reaction is strictly analogous to the preparation of quinaldinic acid and benzaldehyde from quinoline and the same reagents.

B. Reactions involving substituents

Under this heading will be discussed the limited number of reactions directly involving substituents of the isoquinoline molecule and the rather special reactions of the 3,4-dihydroisoquinolines. The latter are obtained directly in the Bischler–Napieralski synthesis and are in general the most readily accessible of the entire group. They may be dehydrogenated to isoquinolines by heating with palladium at about 180°C. (96).

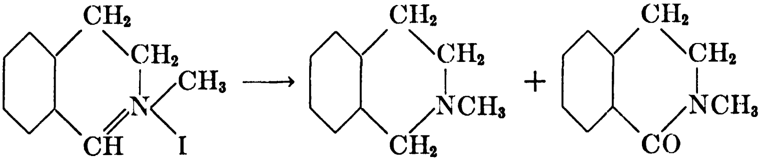
The hydroxyisoquinolines with the hydroxyl group in the pyridine nucleus are soluble in alkali but in general not soluble in dilute acids. The hydroxyl is replaceable by chlorine by means of phosphorus oxychloride (42). When there are two hydroxyls, either one or both of them may be replaced by chlorine (40).

The chloro compounds react with sodium methylate, yielding the corresponding methyl ethers, and heating with hydrogen iodide and phosphorus yields isoquinolines (2). The hydroxy compounds when heated with zinc dust in an atmosphere of hydrogen yield isoquinolines (42). When 1,4-dihydroxy-3-methylisoquinoline is heated with hydrogen iodide and phosphorus to 180°C., there is formed 3-methylisocarbostyril. At 240°C. the second hydroxyl is reduced and 3-methylisoquinoline is obtained (40).

Mills and Smith (74), in the course of their studies of the reactivity of methyl groups in heterocyclic bases, condensed 1-methylisoquinoline with benzaldehyde and obtained 1-styrylisoquinoline. Attempts to obtain 3-styrylisoquinoline from 3-methylisoquinoline failed. There seems to be no record of attempts to condense these methylisoquinolines with the other reagents which have been successfully condensed with quinaldine and with lepidine (i.e., chloral, phthalic anhydride, etc.).

Isoquinoline and its derivatives react with alkyl halides, yielding alkylisoquinolinium halides (16, 59). These halides are for the most part soluble in water and yield on treatment with excess fixed alkali insoluble oily precipitates of uncertain structure. Oxidation with potassium ferricyanide in alkaline solution yields *N*-alkylisocarbostyrils.

Of special interest are the reactions of 3,4-dihydroisoquinoline with a number of reagents. The corresponding methiodide (I) on treatment with alkali undergoes a reduction–oxidation dismutation which is strictly analogous to the Cannizzaro reaction. The products are *N*-methyltetrahydroisoquinoline (II) and the corresponding 1-keto derivative (III) (87). The reaction is of importance in the chemistry of the phthalide-isoquinoline alkaloids. The latter on mild oxidation yield compounds analogous to compound I with alkoxyl groups in the benzene nucleus. The corresponding bases are obtained by adding an excess of a strong alkali to an aqueous solution of the salt. Two of the formulas which have been proposed for the free bases IV and V have received wide recognition.



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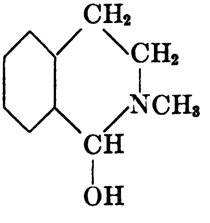
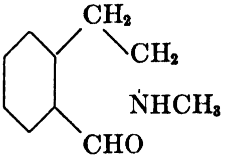
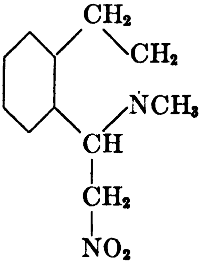


IV



V

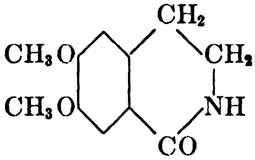


VI

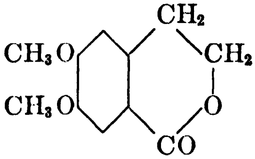
The former (IV) accounts for the ease with which these compounds condense with a variety of reagents. With nitromethane the product has formula VI (60), and the reaction product with meconine or with a substituted meconine and cotarnine are stereoisomers of narcotine (60). Formula V accounts adequately for the Cannizzaro reaction noted above.

Of special importance in alkaloid syntheses is the reduction of methiodides of dihydroisoquinolines. This is readily accomplished by a variety of reducing agents, of which zinc and acetic or hydrochloric acid is one of the most convenient. The products are *N*-methyltetrahydroisoquinolines and it is in this form that most of the isoquinoline alkaloids occur in nature.

Corydaldine (i.e., 6,7-dimethoxytetrahydroisocarbostyril; VII) is obtainable by oxidizing corydaline (25). It yields a crystalline *N*-nitroso derivative which on treatment with alkali is converted into the lactone (VIII).



VII
Corydaldine



VIII

REFERENCES

(1) Andersag, H.: Chem. Abstracts **29**, 6600 (1935).
(2) Bain, D., Perkin, W. H., and Robinson, R.: J. Chem. Soc. **105**, 2392 (1914).
(3) Bamberger, E., and Dieckmann, W.: Ber. **26**, 1217 (1893).
(4) Bamberger, E., and Frew, W.: Ber. **27**, 198 (1894).
(5) Bamberger, E., and Goldschmidt, C.: Ber. **27**, 1954 (1894).
(6) Basu, U.: J. Indian Chem. Soc. **8**, 319 (1931).
(7) Basu, U., and Banerjee, B.: Ann. **516**, 243 (1935).
(8) Bergstrom, F. W., and McAllister, S. H.: J. Am. Chem. Soc. **52**, 2845 (1930).
(9) Bethmann, F.: Ber. **32**, 1104 (1899).
(10) Bischler, A., and Napieralski, B.: Ber. **26**, 1903 (1893).
(11) Braun, J. v., and Wirz, K.: Ber. **60**, 102 (1927).
(12) Braun, J. v., and Zobel, F.: Ber. **56**, 2151 (1923).
(13) Bruckner, V., and Krámli, A.: J. prakt. Chem. **145**, 291 (1936).
(14) Burstin, H.: Monatsh. **34**, 1443 (1913).
(15) Chichibabin, A. E., and Oparina, M. P.: J. Russ. Phys. Chem. Soc. **50**, 543 (1920).
(16) Claus, A., and Edinger, A.: J. prakt. Chem. **38**, 491 (1888).
(17) Claus, A., and Hoffmann, K.: J. prakt. Chem. **47**, 252 (1893).
(18) Claus, A., and Raps, G.: J. prakt. Chem. **45**, 241 (1892).
(19) Claus, A., and Seelemann, A.: J. prakt. Chem. **52**, 1 (1895).
(20) Decker, H., and Becker, P.: Ann. **382**, 369 (1911).
(21) Decker, H., and Becker, P.: Ann. **395**, 342 (1913).
(22) Decker, H., and Kropp, W.: Ber. **42**, 2075 (1909).
(23) Decker, H., Kropp, W., Hoyer, H., and Becker, P.: Ann. **395**, 299 (1913).
(24) Dieckmann, W., and Meiser, W.: Ber. **41**, 3253 (1908).
(25) Dobbie, J. J., and Lauder, A.: J. Chem. Soc. **75**, 673 (1899).
(26) Edinger, A., and Bossung, E.: J. prakt. Chem. **43**, 190 (1891).
(27) Fischer, E.: Ber. **26**, 764 (1893).

(28) Fischer, H., and Krollpfeiffer, F.: Z. physiol. Chem. **82**, 266 (1912).
(29) Findeklee, W.: Ber. **38**, 3542 (1905).
(30) Fritsch, P.: Ber. **26**, 419 (1893).
(31) Fritsch, P.: Ann. **286**, 1 (1895).
(32) Gabriel, S.: Ber. **18**, 1251, 3470 (1885).
(33) Gabriel, S.: Ber. **18**, 2433 (1885).
(34) Gabriel, S.: Ber. **19**, 830 (1886).
(35) Gabriel, S.: Ber. **19**, 1653 (1886).
(36) Gabriel, S.: Ber. **19**, 2354 (1886).
(37) Gabriel, S.: Ber. **20**, 2499 (1887).
(38) Gabriel, S.: Ber. **44**, 70 (1911).
(39) Gabriel, S.: Ber. **49**, 1608 (1916).
(40) Gabriel, S., and Colman, J.: Ber. **33**, 980 (1900).
(41) Gabriel, S., and Colman, J.: Ber. **33**, 2630 (1900).
(42) Gabriel, S., and Neumann, A.: Ber. **25**, 3563 (1892).
(43) Gabriel, S., and Posner, T.: Ber. **27**, 827 (1894).
(44) Goldschmidt, C.: Ber. **27**, 2795 (1894).
(45) Goldschmidt, C.: Ber. **28**, 818 (1895).
(46) Goldschmidt, C.: Ber. **28**, 986 (1895).
(47) Goldschmiedt, G.: Monatsh. **9**, 675 (1888).
(48) Gottlieb, J.: Ber. **32**, 958 (1899).
(49) Graebe, C., and Pictet, A.: Ann. **247**, 304 (1888).
(50) Guareschi, I.: Chem. Zentr. **1896**, **I**, 601.
(51) Hahn, G., and Schales, O.: Ber. **68**, 24, 1310 (1935).
(52) Hahn, G., and Schales, O.: Ber. **69**, 622 (1936).
(53) Hamilton, E. E. P., and Robinson, R.: J. Chem. Soc. **109**, 1029 (1916).
(54) Heilmann, E.: Ber. **23**, 3157 (1890).
(55) Helfer, L.: Helv. Chim. Acta **6**, 789 (1923).
(56) Henry, T. A.: *The Plant Alkaloids.* J. and A. Churchill, Ltd., London (1939).
(57) Hollins, C.: *Synthesis of Nitrogen Ring Compounds.* Ernest Benn, Ltd., London (1924).
(58) Hoogewerff, S., and van Dorp, W. A.: Rec. trav. chim. **4**, 285 (1885).
(59) Hoogewerff, S., and van Dorp, W. A.: Rec. trav. chim. **5**, 305 (1886).
(60) Hope, E., and Robinson, R.: J. Chem. Soc. **105**, 2085 (1914).
(61) Hopkins, F. G., and Cole, S. W.: J. Physiol. **29**, 451 (1903).
(62) Jeiteles, B.: Monatsh. **15**, 807 (1894).
(63) Kaufmann, A., and Dürst, N.: Ber. **50**, 1630 (1917).
(64) Kindler, K., and Brandt, E.: Arch. Pharm. **273**, 478 (1935).
(65) Kindler, K., and Peschke, W.: Arch. Pharm. **269**, 74, 581 (1931).
(66) Kindler, K., and Peschke, W.: Arch. Pharm. **271**, 431 (1933).
(67) Knoevenagel, E., and Fries, A.: Ber. **31**, 767 (1898).
(68) Kolshorn, E.: Ber. **37**, 2474 (1904).
(69) Kusel, H.: Ber. **37**, 1971 (1904).
(70) LeBlanc, M.: Ber. **21**, 2299 (1888).
(71) Lieck, A.: Ber. **38**, 3853 (1905).
(72) Manske, R. H. F., and Robinson, R.: J. Chem. Soc. **1927**, 240.
(73) Meisenheimer, J.: Ber. **59**, 1848 (1926).
(74) Mills, W. H., and Smith, J. L. B.: J. Chem. Soc. **121**, 2724 (1922).
(75) Mohunta, L. M., and Ray, J. N.: J. Chem. Soc. **1934**, 1263.
(76) Perkin, W. H., and Robinson, R.: J. Chem. Soc. **105**, 2376 (1914).
(77) Perkin, W. H., and Robinson, R.: J. Chem. Soc. **115**, 967 (1919).
(78) Pictet, A.: Ber. **38**, 1946 (1905).
(79) Pictet, A., and Gams, A.: Ber. **42**, 2943 (1909).
(80) Pictet, A., and Gams, A.: Ber. **43**, 2384 (1910).

(81) Pictet, A., and Finkelstein, M.: Ber. **42**, 1979 (1909).
(82) Pictet, A., and Kay, F. W.: Ber. **42**, 1973 (1909).
(83) Pictet, A., and Popovici, S.: Ber. **25**, 733 (1892).
(84) Pictet, A., and Spengler, T.: Ber. **44**, 2030 (1911).
(85) Pomeranz, C.: Monatsh. **14**, 116 (1893).
(86) Pomeranz, C.: Monatsh. **15**, 299 (1894).
(87) Pyaman, F. L.: J. Chem. Soc. **95**, 1260 (1909).
(88) Ranedo, J., and Vidol, A.: Anales soc. españ. fís. quím. **28**, 76 (1930).
(89) Reissert, A.: Ber. **38**, 3426 (1905).
(90) Rügheimer, L.: Ber. **19**, 1172 (1886).
(91) Rügheimer, L., and Schön, P.: Ber. **42**, 2374 (1909).
(92) Schales, O.: Ber. **68**, 1579 (1935).
(93) Schneider, W., and Schroeter, K.: Ber. **53**, 1459 (1920).
(94) Schöpf, C., and Bayerle, H.: Ann. **513**, 190 (1934).
(95) Späth, E., and Dobrowsky, A.: Ber. **58**, 1274 (1925).
(96) Späth, E., and Polgar, N.: Monatsh. **51**, 190 (1929).
(97) Staub, P.: Helv. Chim. Acta **5**, 888 (1922).
(98) Sugasawa, S., and Yoshikawa, H.: J. Chem. Soc. **1933**, 1583.
(99) Ulrich, A.: Ber. **37**, 1685 (1904).
(100) Weermann, R. A., and Jongkees, W. J. A.: Rec. trav. chim. **25**, 238 (1906).
(101) Wölbling, H.: Ber. **38**, 3845 (1905).
(102) Zelinsky, N.: Ber. **20**, 922 (1887).

COMMUNICATION TO THE EDITOR

Sir:

Twelve papers were presented at the Symposium on the Thermodynamics of Electrolytic Dissociation which was held under the auspices of the Division of Physical and Inorganic Chemistry at the 101st Meeting of the American Chemical Society in St. Louis, Missouri, on April 8, 1941. Because of the time demanded by the many duties imposed by war conditions it has proved impossible to submit all the papers for publication at this time. It is fortunate indeed that the authors of five of the papers have been able to spare sufficient time to prepare them for publication. We hope that the authors of the other papers will be able in the near future to submit them to the Editor of this journal; perhaps a second group can appear together.

I wish to express my gratitude to all the participants in the symposium for the general excellence of their contributions; to Professor John G. Kirkwood who, as chairman of the Division of Physical and Inorganic Chemistry, recognized the need for the discussion of the subject and furnished the opportunity for a symposium; and to the editors of *Chemical Reviews* who have offered unlimited coöperation in spite of our failure in this difficult year to adhere to the publication schedule planned.

T. F. Young.

Department of Chemistry
University of Chicago
Chicago, Illinois
March 15, 1942.

ERRATA

Volume 28, Number 3, June, 1941

Page 552: Equation 37 should read

$$\log L = a + bT_c$$

Volume 29, Number 3, December, 1941

Page 472: In figure 2 the solid lines designate systems in salt water, and the broken lines represent systems in pure water. The legend should read as follows: "Comparison of the effects of pressure upon ionic equilibria in salt water (————) and in pure water (— — —) at 25°C."

Volume 30, Number 1, February, 1942

Page 109: Reference 81 should read "Schultz, F.: Chem.-Ztg. **32**, 55, 729 (1908)."

THE EFFECT OF DIELECTRIC CONSTANT ON ACID-BASE EQUILIBRIA[1, 2]

MARTIN KILPATRICK

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Received February 10, 1942

Acid-base equilibria are usually represented by the equilibrium constant for the reaction

$$A^n + S \rightleftharpoons SH^+ + B^{n-1} \tag{1}$$

where A is an acid, S the solvent, SH^+ the solvated proton, and B the conjugate base. The change in the value of the equilibrium constant with electrolyte concentration depends upon the charge types involved and the solvent. For dilute aqueous solutions the concentration of solvent is usually considered constant, and the equilibrium constant is written

$$K_C = C_{H_3O^+}\frac{C_B}{C_A} \tag{2}$$

For the monosubstituted benzoic acids

$$\frac{K_C\ (\mu = 0.05 \text{ for solvent salt LiCl})}{K_C\ (\mu = 0)}$$

is found to be 1.45 in water, 5.5 in methyl alcohol, approximately 17 in ethyl alcohol, and 7.0 for a dioxane-water mixture of the same dielectric constant as ethyl alcohol. It has been reported that log K_C varies linearly with the reciprocal of the dielectric constant for glycerol-water and dioxane-water mixtures.

Instead of using the solvated proton as the standard acid to compare acid-base equilibrium constants in various solvents, it is more convenient to refer to a standard of the same charge type. This equilibrium constant $K_{A_xB_0}$ for the reaction

$$A_x + B_0 \rightleftharpoons A_0 + B_x \tag{3}$$

(where A_0 is benzoic acid for substituted benzoic acids and acetic acid for the aliphatic acids) is the ratio of the two dissociation constants as given in equation 2. $K_{A_xB_0}$ is approximately independent of the concentration of solvent salt over a considerable range in the solvents water, ethylene glycol, and methyl, ethyl, *n*-propyl, and *n*-butyl alcohols. The change in the constant $K_{A_xB_0}$ with dielectric constant can be represented for the pure solvent by the equation

$$\log K_{A_xB_0} = \log K_{a_xb_0} + \frac{L}{D} \tag{4}$$

where L is a constant specific to each acid. For the mixed solvents such as dioxane-water and alcohol-water, linearity with the reciprocal of the dielectric constant is not observed.

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[1] Presented at the Symposium on the Thermodynamics of Electrolytic Dissociation, which was held under the auspices of the Division of Physical and Inorganic Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Missouri, April, 1941.

[2] The author would like to make due acknowledgment of two grants from the Penrose Fund of the American Philosophical Society for the study of relative acid strengths in aqueous and non-aqueous solutions, and of a grant from the Faculty Research Committee of the University of Pennsylvania.

Acid–base equilibria are often represented by the equilibrium constant for the reaction

$$\mathrm{A}_x^n + \mathrm{S} \rightleftharpoons \mathrm{SH}^+ + \mathrm{B}_x^{n-1} \tag{1}$$

where A_x is an acid, S a basic solvent, SH^+ the solvated proton, B_x the conjugate base, and n the charge on the acid. This process involves a number of steps. When an uncharged acid A_x is introduced into the solvent S, the molecules of solvent orient themselves around the acid molecules under the influence of dipole or other forces; a proton is transferred from the acid A_x to the base S, and two ions, the solvated proton and the base B_x^-, are formed. In addition to this, the solvent molecules are now oriented about the ions and ionic atmospheres created. The equilibrium constant for such a process is given by the equation

$$K_{\mathrm{A}_x\mathrm{S}} = \frac{C_{\mathrm{SH}^+}}{C_\mathrm{S}} \cdot \frac{C_{\mathrm{B}_x^-}}{C_{\mathrm{A}_x}} \tag{2}$$

where the concentrations may be expressed in moles per liter, moles per 1000 grams of solvent, or mole fractions.

TABLE 1

Effect of electrolyte concentration on the reaction $\mathrm{A}_0 + \mathrm{S} \rightleftharpoons \mathrm{SH}^+ + \mathrm{B}_0^-$

Solvent	Water	Methyl alcohol	Ethyl alcohol	Dioxane–water
Dielectric constant	78.5	31.5	24.2	25
$\dfrac{K_C\ (\mu = 0.05,\ \text{mostly LiCl})}{K_C\ (\mu = 0)}$	1.45	5.5	18	7

Since the concentration of solvent is considered constant, it is customary to write

$$C_\mathrm{S} \cdot K_{\mathrm{A}_x\mathrm{S}} = C_{\mathrm{SH}^+} \frac{C_{\mathrm{B}_x^-}}{C_{\mathrm{A}_x}} = K_C \tag{3}$$

where K_C is the familiar dissociation constant. Both $K_{\mathrm{A}_x\mathrm{S}}$ and K_C vary with ionic strength, the change in sufficiently dilute solutions for solvents of sufficiently high dielectric constant being given by the relation

$$\log K_C = \log\,[K_C]^0 + A\sqrt{\mu} \tag{4}$$

where A/2 is the familiar Debye–Hückel constant. The values for A at 25°C. are 1.020 for water (dielectric constant = 78.54), 4.02 for methyl alcohol (D = 31.5), 5.97 for ethyl alcohol (D = 24.2), and 9.79 for butyl alcohol (D = 17.4). For more concentrated solutions no theoretical equation can be given, but it might be well to point out that the change in the equilibrium constant K_C is of considerable magnitude and depends on the solvent (10); see table 1.

Leaving the correction for the ionic atmosphere, it is seen that the solvent itself and the dielectric constant of the medium play important rôles in the process represented by equation 1. The formation of the ions involves a charging process, the transfer of the proton involves electrostatic work depending on the dielectric constant, and the solvation of the ions and molecules involves the dielectric constant and the structure of the solvent (4).

Equation 3 makes the standard acid the solvated proton, and equation 2 represents the ratio of the dissociation constant of the acid A_x to that of the acid SH^+. Since the solvated proton is a different acid in each basic solvent, one can hope to eliminate the solvent by choosing a standard acid other than the solvated proton (35). For example, if we choose benzoic acid as the standard acid, we have

$$K_C = C_{SH^+} \frac{C_{B_0^-}}{C_{A_0}} \tag{5}$$

and, dividing equation 3 by equation 5,

$$\frac{K_{C(A_x)}}{K_{C(A_0)}} = \frac{C_{B_x^-}}{C_{A_x}} \cdot \frac{C_{A_0}}{C_{B_0^-}} = K_{A_xB_0} \tag{6}$$

which is the equilibrium constant for the reaction

$$A_x + B_0^- \rightleftharpoons A_0 + B_x^- \tag{7}$$

This equilibrium constant will be less sensitive to changes in ionic strength, since there are equal numbers of equally charged ions on both sides. The effect of the nature of the solvent will be much less, and the rôles of the dielectric constant of the medium and the structure of the acids will be relatively more important. In any case, table 2 shows that the order of acid strength is not independent of the medium (6, 7, 8, 9, 10, 22, 23). In table 2 the order of acid strength decreases from top to bottom. The order will be the same in any particular solvent, whether we use the solvated proton or any other acid as the standard acid. The practice of using the thermodynamic dissociation constant in water at 25°C. as a basis of acid strength has been defended by Dippy (5), who claims that there is no proof that the dissociation constants in water are likely to give an ambiguous relative order. He recognizes exceptions in the case of the ortho-substituted benzoic acids. Since the equations

$$-RT \ln K^0 = \Delta F^0 = \Delta H_0 - T\Delta S^0 \tag{8}$$

and

$$\frac{d(\Delta H)}{dT} = \Delta C_p \tag{9}$$

connect the equilibrium constant, the free-energy change, the heat content change, the entropy change, and the change in heat capacity, a relationship between change of structure and any of these thermodynamic quantities might be sought. Harned and Embree (18) point out that in any discussion of dissociation constants in relation to constitution it will be essential to compare the values of the dissociation constants at their maxima or at their corresponding temperatures θ as given by the equation

$$\log K = \log K_m - p(t - \theta)^2 \tag{10}$$

TABLE 2

Order of acid strengths of substituted benzoic acids

SOLVENT		H$_2$O	(CH$_2$OH)$_2$	CH$_3$OH	C$_2$H$_5$OH	*n*-C$_3$H$_7$OH	*n*-C$_4$H$_9$OH	DIOXANE-WATER		
DIELECTRIC CONSTANT	∞ Extrapolated	78.5	37.7	31.5	24.2	20.1	17.4	55	40	15
Substituent groups	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-NO$_2$	*o*-OH
	o-I	*o*-Br	*o*-OH	*o*-OH	*o*-OH	*o*-OH	*o*-OH	*o*-OH	*o*-OH	*o*-NO$_2$
	o-Br	*o*-I	*o*-Br	*o*-Br	*m*-NO$_2$	*o*-Br	*p*-NO$_2$	*o*-Br	*p*-NO$_2$	*p*-NO$_2$
	o-Cl	*o*-Cl	*o*-Cl	*o*-Cl	*p*-NO$_2$	*m*-NO$_2$	*m*-NO$_2$	*o*-Cl	*o*-Br	*m*-NO$_2$
	o-OH	*o*-OH	*o*-I	*o*-I	*o*-Br	*p*-NO$_2$	*o*-Br	*o*-I	*m*-NO$_2$	*o*-F
	o-F	*o*-F	*p*-NO$_2$	*m*-NO$_2$	*o*-Cl	*o*-I	*o*-Cl	*o*-F	*o*-Cl	*o*-Br
	p-NO$_2$	*p*-NO$_2$	*m*-NO$_2$	*p*-NO$_2$	*o*-I	*o*-Cl	*o*-I	*p*-NO$_2$	*o*-F	*o*-Cl
	m-NO$_2$	*m*-NO$_2$	*o*-F	*o*-F	*o*-F	*o*-F	*o*-F	*m*-NO$_2$	*o*-I	*o*-I
	o-CH$_3$	*m*-Br	*m*-Br	*m*-Br	*m*-Br	*m*-Br	*m*-Cl	*m*-Cl	*m*-Cl	*m*-Cl
	m-Br	*m*-Cl	*m*-Cl	*m*-Cl	*m*-Cl	*m*-I	*m*-Br	*m*-Br	*m*-Br	*m*-Br
	m-Cl	*m*-I	*m*-I	*m*-I	*m*-I	*m*-Cl	*m*-I	*m*-F	*m*-F	*m*-F
	m-F	*m*-F	*m*-F	*m*-F	*m*-F	*m*-F	*p*-Br	*m*-I	*m*-I	*m*-I
	m-I	*o*-CH$_3$	*p*-Br	*p*-Br	*p*-Br	*p*-I	*m*-F	*p*-F	*p*-Cl	*p*-Br
	m-OH	*p*-Br	*p*-I	*p*-Cl	*p*-I	*p*-Br	*p*-I	*o*-CH$_3$	*p*-I	*p*-Cl
	p-I	*p*-Cl	*p*-Cl	*p*-I	*p*-Cl	*p*-Cl	*p*-Cl	*m*-OH	*p*-Br	*p*-I
	p-Br	*o*-OCH$_3$	*o* OCH$_3$	*p*-F	*o*-OCH$_3$	*o*-OCH$_3$	*o*-OCH$_3$		*p*-F	*p*-F
	p-Cl	*m*-OH	*o*-CH$_3$	*o*-OCH$_3$	*p*-F	*p*-F	*p*-F			
	o-OCH$_3$	*p*-F	*p*-F	*o*-CH$_3$	*o*-CH$_3$	*o*-CH$_3$	*o*-CH$_3$			
Benzoic acid										
Substituent groups	*p*-F	*m*-CH$_3$	*m*-OH	*m*-CH$_3$	*m*-CH$_3$	*m*-CH$_3$	*m*-CH$_3$	*o*-OCH$_3$	*m*-OH	*m*-OH
	m-CH$_3$	*p*-CH$_3$	*m*-CH$_3$	*m*-OH	*m*-OH	*m*-OH	*m*-OH	*m*-CH$_3$	*o*-CH$_3$	*o*-OCH$_3$
	p-CH$_3$	*p*-OCH$_3$	*p*-CH$_3$	*p*-CH$_3$	*p*-CH$_3$	*p*-CH$_3$	*p*-CH$_3$	*p*-CH$_3$	*o*-OCH$_3$	*m*-CH$_3$
	p-OCH$_3$	*p*-OH	*p*-OCH$_3$	*p*-OCH$_3$	*p*-OCH$_3$	*p*-OCH$_3$	*p*-OCH$_3$	*p*-OCH$_3$	*m*-CH$_3$	*p*-CH$_3$
	p-OH		*p*-OH	*p*-OH	*p*-OH	*p*-OH	*p*-OH	*p*-OH	*p*-CH$_3$	*o*-CH$_3$
									p-OCH$_3$	*p*-OCH$_3$
									p-OH	*p*-OH

where K_m is the dissociation constant at the maximum and θ the temperature of the maximum. The constant p was thought to be the same for many acids, but Walde (32) pointed out that log K is not a quadratic function of the temperature, and Harned and Robinson (20) state that p is a constant characteristic of each acid. More recently it has been shown that the properties of the solvent (water) almost completely determine the value of p (14, 28).

Everett and Wynne-Jones (13) show that the order is not always the same for the different thermodynamic functions; for example, for the toluic acids in water the order for $\Delta F_{298°}$ is

o-toluic > benzoic > *m*-toluic > *p*-toluic

while ΔH_0 gives

o-toluic > *m*-toluic > *p*-toluic ~ benzoic

It is also to be noted from table 2 that all three toluic acids are weaker than benzoic acid in dioxane-water mixtures of dielectric constants 40 and 15.

The effect of temperature on the relative strengths of carboxylic acids has been treated by several authors (2, 14, 15, 16), and a correlation of the two effects

I	II
$\left(\frac{\mathrm{d} \ln K_{A_xB_0}}{\mathrm{d}D}\right)_{\text{Temperature shift}}$	$\left(\frac{\mathrm{d} \ln K_{A_xB_0}}{\mathrm{d}D}\right)_{\text{Solvent shift}}$
Same solvent	Same temperature

has been attempted. However, the two effects are not the same and should not be so considered. Following Baughan (2), for a given solvent,

$$\frac{\mathrm{d}}{\mathrm{d}T}[\ln K_{A_xB_0}] = \frac{\mathrm{d}}{\mathrm{d}T}\left[\frac{-\Delta F}{RT}\right] = \frac{\Delta H}{RT^2}$$

$$= \frac{1}{RT^2}\left\{[(\Delta H_x)_{A_x} - (\Delta H_x)_{A_0}] + \frac{e^2}{2D}\left(\frac{1}{r_{B_x^-}} - \frac{1}{r_{B_0^-}}\right)\left[1 + T\frac{\partial \ln D}{\partial T}\right]\right\} \tag{11}$$

where $r_{B_x^-}$ and $r_{B_0^-}$ are the radii of the two ions in equation 7, e is the charge on the ion, D is the dielectric constant of the medium, and $(\Delta H_x)_{A_x}$ and $(\Delta H_x)_{A_0}$ are the temperature-independent parts of the heats of reaction for the process represented by equation 1. Now for a given solvent we can regard D as a function of T, or *vice versa*. If we choose D as the independent variable, we write

$$\frac{\mathrm{d} \ln K_{A_xB_0}}{\mathrm{d}D} = \frac{\partial \ln K_{A_xB_0}}{\partial D} + \frac{\partial \ln K_{A_xB_0}}{\partial T}\frac{\mathrm{d}T}{\mathrm{d}D} \tag{12}$$

Now

$$\frac{\mathrm{d} \ln K_{A_xB_0}}{\mathrm{d}T} = \frac{\Delta H}{RT^2} \tag{13}$$

where

$$\frac{\mathrm{d}\ln K_{\mathrm{A}_x\mathrm{B}_0}}{\mathrm{d}T} \neq \frac{\partial \ln K_{\mathrm{A}_x\mathrm{B}_0}}{\partial T} \tag{14}$$

in equation 12.

Since (3)

$$\Delta F_{\mathrm{el}} = \frac{e^2}{2D}\left(\frac{1}{r_{\mathrm{B}_x^-}} - \frac{1}{r_{\mathrm{B}_0^-}}\right) \tag{15}$$

and

$$\overset{\mathrm{I}}{\left(\frac{\mathrm{d}\ln K_{\mathrm{A}_x\mathrm{B}_0}}{\mathrm{d}D}\right)_{\text{Temperature effect}}} = \frac{\Delta F_{\mathrm{el}}}{RTD} + \frac{\partial}{\partial T}\left(\frac{-\Delta F}{RT}\right)\frac{\mathrm{d}T}{\mathrm{d}D} \tag{16}$$

and

$$\overset{\mathrm{II}}{\left(\frac{\mathrm{d}\ln K_{\mathrm{A}_x\mathrm{B}_0}}{\mathrm{d}D}\right)_{\text{Solvent effect}}} = \frac{\Delta F_{\mathrm{el}}}{RTD} \tag{17}$$

the criterion that I $=$ II is not that $\Delta H = 0$, but that

$$\frac{\partial}{\partial T}\left(\frac{-\Delta F}{RT}\right)\frac{\mathrm{d}T}{\mathrm{d}D} = 0 \tag{18a}$$

or

$$\frac{1}{RT^2}\left\{[(\Delta H_x)_{\mathrm{A}_x} - (\Delta H_x)_{\mathrm{A}_0}] + \frac{e^2}{2D}\left(\frac{1}{r_{\mathrm{B}_x^-}} - \frac{1}{r_{\mathrm{B}_0^-}}\right)\right\}\frac{\mathrm{d}T}{\mathrm{d}D} = 0 \tag{18b}$$

If $\mathrm{d}T/\mathrm{d}D = 0$ (which means that the dielectric constant and temperature of a solvent are independent of each other), or in the event that the { } bracket in equation 18b is equal to zero, then effect I will be equal to effect II. But since $\mathrm{d}T/\mathrm{d}D \neq 0$ for most solvents, and since only one value of the dielectric constant satisfies the requirement that the { } bracket in equation 18b be equal to zero, it is evident that, in general, effects I and II will be unequal.

Baughan has shown that the variation with temperature of the heats of dissociation of a number of acids in water conforms to an equation similar to 11. However, it is to be noted that the mean radii of the ions used in the calculations are very small, being less than 1 Å. in most cases. Moreover, the substituted acetic acids have smaller radii than acetic acid. On the other hand, calculations for effect II from the equation

$$\overset{\mathrm{II}}{\left(\frac{\mathrm{d}\ln K_{\mathrm{A}_x\mathrm{B}_0}}{\mathrm{d}D}\right)_T} = \frac{1}{RTD}\left(\frac{e^2}{2D}\left[\frac{1}{r_{\mathrm{B}_x^-}} - \frac{1}{r_{\mathrm{B}_0^-}}\right]\right) \tag{19}$$

yield values of the radii which are much larger (21). It is evident that the radii should not be considered to have any physical significance but should be regarded as adjustable parameters in the equation. It is generally recognized that the Born equation (3) for the work done to create the field due to charges on two ions of radii r_+ and r_- in a medium of dielectric D

$$W = \frac{e^2}{2D}\left(\frac{1}{r_+} + \frac{1}{r_-}\right) \tag{20}$$

is only an approximation. Everett and Wynne-Jones (15) have recognized the inadequacy of the Born equation to account for ΔC_p for the dissociation of acids, and Everett and Coulson (12) have attempted to evaluate the contribution of the orientation of the solvent to ΔC_p.

ACID STRENGTHS IN PURE SOLVENTS

In a comparison of the colorimetric and E.M.F. methods for the determination of the equilibrium constant of the reaction of equation 7, it was found that for the solvent methyl alcohol the equilibrium constant was essentially the same at zero ionic strength as at $\mu = 0.05$ (mostly lithium chloride). Figure 1 shows that for the aliphatic acids (standard acid, acetic acid) there is no marked change in the value of the equilibrium constant, $K_{A_xB_0}$, over a considerable range of electrolyte concentration if the acids are similar and do not contain strongly polar groups. Examination of the literature shows that these examples are typical (24, 25, 26).

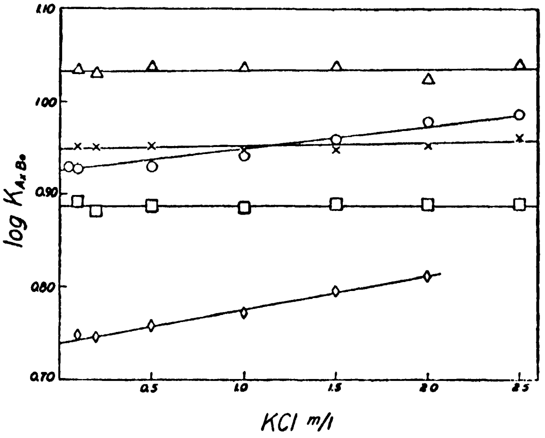

FIG. 1. Effect of electrolyte concentration. Standard acid, acetic acid. △, formic acid; ×, *n*-butyric acid (up 1.0); ○, monochloroacetic acid (down 1.0); □, propionic acid (up 1.0); ◇, trimethylacetic acid (up 1.0).

The results of the determination of the ratio of the dissociation constants of the substituted benzoic acids to the dissociation constant of benzoic acid in the solvents water, ethylene glycol, methyl alcohol, and ethyl alcohol can be represented by the equation

$$\log K_{A_xB_0} = \log K_{a_xb_0} + L\left(\frac{1}{D}\right) \tag{21}$$

where $K_{a_x b_0}$ is the intercept at $1/D = 0$; it has been called the "intrinsic acid strength" by Wynne-Jones (35). It is assumed that the intrinsic acid strengths are independent of solvent. Figure 2 presents a few typical results. An examination of the results for the twenty-three acids shows that equation 21 holds in all the pure solvents of dielectric constant > 24, the only exceptions being the ortho acids in the solvent ethylene glycol. Table 3 gives the slopes and intercepts of equation 21, as determined by the method of least squares for all the experimental results in the four solvents (5, 7, 8, 9, 10, 22, 23).

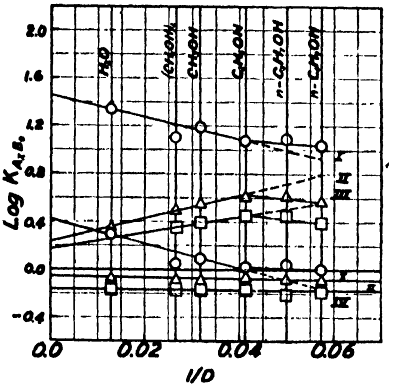

FIG. 2

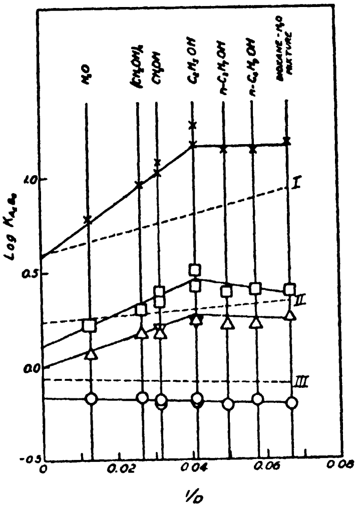

FIG. 3

FIG. 2. Effect of substituents upon the acid strength of benzoic acid. Curve I, *o*-iodobenzoic acid; curve II, *m*-iodobenzoic acid; curve III, *p*-iodobenzoic acid; curve IV, *o*-methylbenzoic acid; curve V, *m*-methylbenzoic acid; curve VI, *p*-methylbenzoic acid.

FIG. 3. Kirkwood-Westheimer model. Curve I, *p*-nitrobenzoic acid; curve II, *p*-chloro- and *p*-fluoro-benzoic acids; curve III, *p*-methylbenzoic acid. Experimental values: ×, *p*-nitrobenzoic acid; □, *p*-chlorobenzoic acid; △, *p*-fluorobenzoic acid; ○, *p*-methylbenzoic acid.

Eucken (11) and Schwarzenbach and Egli (31) have given a formula for the electrostatic effect of a substituent on the acid strength, of the form

$$\log K_{A_xB_0} = \frac{-em \cos\theta}{2.303kTD_E r^2} \tag{22}$$

where m is the dipole moment of the carbon-substituent bond (taken from the corresponding substituted benzene), θ the angle between the dipole and the line joining the mid-point of the dipole to the proton, r the distance from the proton to the mid-point of the dipole, and D_E the effective dielectric constant through which the electrostatic field operates. The difficulties of applying such an equation are that the calculated value of $K_{A_xB_0}$ is very sensitive to the value chosen for r, and that D_E must be evaluated. Kirkwood and Westheimer (34) have evaluated D_E by considering the ions and molecules involved as ellipsoidal cavities of low dielectric constant with the charges along the focal line, and Westheimer (33) has recently evaluated log $K_{A_xB_0}$ for the para-substituted benzoic acids in aqueous solution. The calculations and comparison with experiment for the alcohols as solvent have been given elsewhere (9, 22, 23). Figure 3 shows that the observed relative acid strengths are more sensitive to the macrodielectric constant than the predictions of theory. Calculations are in progress with another model which approximates the benzenoid molecule more closely.[3] In this model the cavities are considered as oblate spheroids with the charges on the focal ring. This model permits calculation of the meta-substituted acids and may reveal the magnitude of the rôle of resonance in the benzene ring.

TABLE 3

Intercepts and slopes of equation 21

SUBSTITUENT	ORTHO		META		PARA	
	log $K_{a_xb_0}$	L	log $K_{a_xb_0}$	L	log $K_{a_xb_0}$	L
NO_2	2.112	−8.9	0.491	17.4	0.575	14.
I	1.442	−8.0	0.225	10.0	0.141	8.2
Br	1.419	−5.5	0.281	9.3	0.128	8.7
Cl	1.342	−4.7	0.280	8.6	0.104	8.5
F	0.889	3.8	0.237	8.0	−0.009	6.5
CH_3	0.376	−8.7	−0.065	−0.4	−0.164	−0.6
OCH_3	0.056	4.0			−0.227	−3.5
OH	0.982	17.1	0.219	−10.	−0.270	−7.1

ACID STRENGTHS IN MIXED SOLVENTS

When the solvent is composed of more than one base the reaction represented by equation 1 may be more complicated, owing to the fact that two bases are competing for the proton and two solvated protons may be formed. In addition to this, the solvent dipoles will be oriented differently around the ions and solute molecules, and in some cases, such as dioxane–water mixtures, there will be an unmixing of the solvent. In dioxane–water mixtures the envelope around the ions is poorer in dioxane than is the bulk of the solution (30). In the earlier work (29) a comparison of the values of $K_{A_xB_0}$ for the solvents ethyl alcohol and dioxane–water mixtures of the same dielectric constant showed quite good agreement, and it was concluded that the dielectric constant was the important factor. Further work (10) in dioxane–water mixtures of dielectric constants 55 and 40 has shown that the results are not in accord with equation 21. A few typical results are presented in figure 4, in which the dotted lines represent the line of equation 21 for the pure solvents. In general, for agreement with the results in pure solvents one would have to consider the dioxane–water mixtures to have lower dielectric constants than the experimental values. These results were obtained by the E.M.F. method and should be checked by the colorimetric.

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[3] These calculations are being carried out by J. N. Sarmousakis at the University of Pennsylvania.

From experimental determinations of the dissociation constant of acetic acid in glycerol–water mixtures of dielectric constant 81.1 to 73.3 at 18°C., Adell (1) concludes that the dissociation constant, expressed in moles per liter of solvent, can be represented by the equation

$$-\log K_0 = 2.878 + \frac{150.1}{D} \qquad (23)$$

Lynch and La Mer (27), from determinations of the dissociation constants of acetic, propionic, butyric, and benzoic acids in dioxane–water mixtures, observe that the logarithm of the dissociation constant (expressed in moles per 1000 g. of solvent) is a linear function of the reciprocal of the dielectric constant from $D = 78$ to $D = 21$. Harned (17) shows that log K for acetic acid is not a linear function of the reciprocal of the dielectric constant, and Harned and Fallon (19) show that the logarithm of the dissociation constant is nearly linear with the mole fraction of dioxane. Quite apart from the question of linearity with the reciprocal of the dielectric constant, it seems preferable to use equation 2 rather than the customary dissociation constant of equation 3. Provided there is only one solvated proton formed (H_3O^+), the actual process is

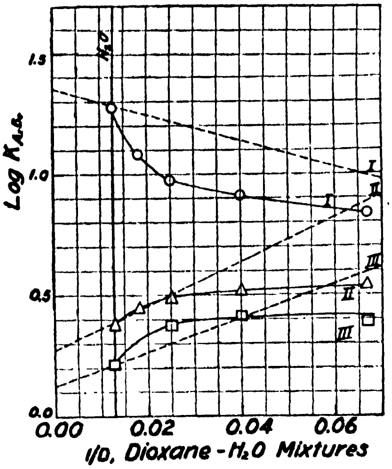

FIG. 4

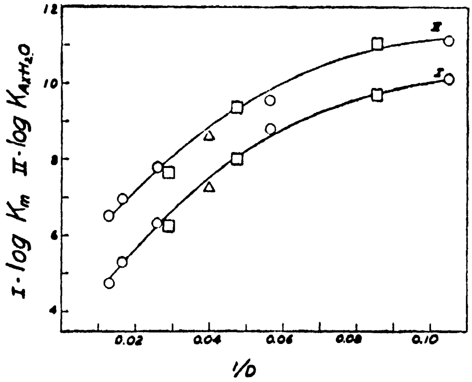

FIG. 5

FIG. 4. Effect of substituents on the acid strength of benzoic acid. Curves I, *o*-chlorobenzoic acid; curves II, *m*-chlorobenzoic acid; curves III, *p*-chlorobenzoic acid.

FIG. 5. Dissociation constant of acetic acid in dioxane–water mixtures. ○, Harned *et al.*, □, Lynch and La Mer; △, Minnick and Kilpatrick.

$$A_z + H_2O \rightleftharpoons H_3O^+ + B_z^- \qquad (24)$$

and the equilibrium constant

$$K_{A_x\cdot H_2O} = \frac{C_{H_3O^+}}{C_{H_2O}}\cdot\frac{C_{B_x}}{C_{A_x}} \qquad (25)$$

takes account of the changing number of moles of water in the solution. In figure 5 a graph of log K_m and log $K_{A_x\cdot H_2O}$ *versus* $1/D$ is presented which indicates the difference in slope if an extrapolation to $1/D = 0$ is attempted.

REFERENCES

(1) Adell, B.: Z. physik. Chem. **A186,** 27 (1940).
(2) Baughan, E. C.: J. Chem. Phys. **7,** 951 (1939).
(3) Born, M.: Z. Physik **1,** 45 (1920).
(4) Coolidge, A. S., and Bent, H. E.: J. Am. Chem. Soc. **58,** 505 (1936).
(5) Dippy, J. F. J.: Chem. Rev. **25,** 151 (1939).
(6) Elliott, J. H.: J. Phys. Chem. **46,** 221 (1942).
(7) Elliott, J. H., and Kilpatrick, M.: J. Phys. Chem. **45,** 454 (1940).
(8) Elliott, J. H., and Kilpatrick, M.: J. Phys. Chem. **45,** 466 (1940).
(9) Elliott, J. H., and Kilpatrick, M.: J. Phys. Chem. **45,** 472 (1940).
(10) Elliott, J. H., and Kilpatrick, M.: J. Phys. Chem. **45,** 485 (1941).
(11) Eucken, A.: Angew. Chem. **45,** 203 (1932).
(12) Everett, D. H., and Coulson, C. A.: Trans. Faraday Soc. **36,** 633 (1940).
(13) Everett, D. H., and Wynne-Jones, W. F. K.: Trans. Faraday Soc. **35,** 1380 (1939).
(14) Gurney, R. W.: J. Chem. Phys. **6,** 499 (1938).
(15) Hammett, L. P.: J. Chem. Phys. **4,** 613 (1936).
(16) Hammett, L. P.: J. Am. Chem. Soc. **59,** 96 (1937).
(17) Harned, H. S.: J. Phys. Chem. **43,** 275 (1939).
(18) Harned, H. S., and Embree, N. D.: J. Am. Chem. Soc. **56,** 1050 (1934).
(19) Harned, H. S., and Fallon, L. D.: J. Am. Chem. Soc. **61,** 2377 (1939).
(20) Harned, H. S., and Robinson, R. A.: Trans. Faraday Soc. **36,** 973 (1940).
(21) Kilpatrick, M.: Trans. Electrochem. Soc. **72,** 95 (1937).
(22) Kilpatrick, M., and Mears, W. H.: J. Am. Chem. Soc. **62,** 3047 (1940).
(23) Kilpatrick, M., and Mears, W. H.: J. Am. Chem. Soc. **62,** 3051 (1940).
(24) Larsson, E., and Adell, B.: Z. physik. Chem. **A156,** 352 (1931).
(25) Larsson, E., and Adell, B.: Z. physik. Chem. **A156,** 381 (1931).
(26) Larsson, E., and Adell, B.: Z. physik. Chem. **A157,** 342 (1931).
(27) Lynch, C. C., and LaMer, V. K.: J. Am. Chem. Soc. **60,** 1252 (1938).
(28) Magee, J. L., Ri, T., and Eyring, H.: J. Chem. Phys. **9,** 419 (1941).
(29) Minnick, L. J., and Kilpatrick, M.: J. Phys. Chem. **43,** 259 (1939).
(30) Scatchard, G.: J. Chem. Phys. **9,** 34 (1941).
(31) Schwarzenbach, G., and Egli, H.: Helv. Chim. Acta **17,** 1183 (1934).
(32) Walde, A. W.: J. Phys. Chem. **39,** 477 (1935).
(33) Westheimer, F. H.: J. Am. Chem. Soc. **61,** 1977 (1939).
(34) Westheimer, F. H., and Kirkwood, J. G.: J. Chem. Phys. **6,** 513 (1938).
(35) Wynne-Jones, W. F. K.: Proc. Roy. Soc. (London) **A140,** 440 (1933).

MOLAL VOLUMES. V

THERMODYNAMIC PROPERTIES OF ELECTROLYTES AT INFINITE DILUTION[1]

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The extent of the validity of Born's equation for the electrical part of the free energy of an ion at infinite dilution is not yet definitely known. Clearly this equation has failed in several respects. While the ionic radius is to be considered merely an empirical parameter, Born's expression might still be useful to relate various thermodynamic properties of solutions of electrolytes at infinite dilution. Subject to this restriction, Born's equation is confirmed within the narrow limits of experimental errors by a comparison of heat capacities with new data on molal volumes of acetic acid. New experimental data confirm the limiting law relating the apparent molal volumes of electrolytes and the concentration.

As old as Arrhenius' theory are the attempts to derive from electrostatics the influence exerted by the electric charges of the ions on their thermodynamic properties. That the dependence of the free energy on concentration differed from the laws of the perfect solution was recognized by Planck and van't Hoff in 1887 (*cf.* 1), and the first quantitative theory of electrostatic interaction was developed by Malmström (23) in 1905. The electric influence of polar substituents on the strength of acids was studied by Wegscheider (38) in 1902. The electric part of the free energy of an ion at infinite dilution, explicitly derived by Born (6), was involved in the derivation of the electrostriction effect (1894) by Drude and Nernst (8). The theories which started from these three points are well known.

Although the electric parts of *all* thermodynamic properties can be derived from Born's equation[3] by straightforward thermodynamics (*cf.* 11), most of the consequences have been discussed only in recent times. The obvious reason of this delay is the uncertainty, from a theoretical viewpoint, of Born's equation itself, and of the additional assumptions required to isolate the electric part of any property.

Webb (37) used a more elaborate model for the purpose of replacing Born's equation. However, Webb uses the classical formula of the inner field, which requires that the Lorentz–Lorenz term does not depend on pressure, a condition not even approximately satisfied by a dipole liquid like water (*cf.* also 3). To expect a good approximation in spite of this simplification would be the less justified, as high pressures are involved in the vicinity of an ion.

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[3] Born's equation represents the electric part of the *free* energy of the solution, not of the energy or heat content. This is to be concluded from Gross and Halpern's discussion (12) of the analogous problem involved in the theory of Debye and Hückel. The equation represents the electric part of the Gibbs free energy as well as of the Helmholtz free energy, the reference states of the solution of discharged ions being different in the two cases (*cf.* 27).

The dipole character of water was taken into account by Ingold (16). However, there is still no way to estimate the effect of the depolymerization of water in the neighborhood of an ion. It must be concluded from Onsager's discussion (25) of the dielectric constant that this effect is essential. An expression for the free energy of an ion in a non-associated dipole solvent probably may be derived from Onsager's theory; however, there still appears no way of a consistent treatment of aqueous solutions.

On the other hand, there is some experimental evidence that Born's equation, in spite of the weakness of its theoretical basis, represents in some respects a good approximation (3, 13, 21). As even a restricted validity will be useful, a thorough experimental test appears to be worthwhile.

Several restrictions must be stated from the start. It can be concluded from the investigations of Webb and Ingold, and it is demonstrated by available experimental data, that the Born ionic radii are far too small and hardly more than arbitrary parameters. The non-electric parts of F, ΔF, H, ΔH, C_p, V (Δ indicating ionization) must not be neglected. Thus, if any, the non-electric parts of only the following properties may be expected to be small compared with the electric terms: ΔC_p, ΔV, and the variation of C_p and V with temperature, pressure, and the composition of the solvent.

Born's equation was first tested by Scatchard (33) with respect to the variation of the free energy with the dielectric constant of the solvent, and similar tests have been carried out by other authors (2, 5, 7, 14, 21, 24, 26, 41). The somewhat diverging results may be summed up in the statement that the influence of the electrostatic term is more or less noticeable but frequently cannot account for the whole change,—not a surprising result as the non-electric part cannot be supposed to be independent of the solvent.

Kritschewsky (20) obtained satisfactory results testing the pressure dependence of the molal volume.

The temperature dependence of the ionization constant, discussed by Gurney (13) and Baughan (3), has been carefully tested in the case of acetic acid by La Mer and Brescia (21). This test is equivalent to a comparison of the ionic radii calculated from ΔC_p and the variation of this quantity with temperature.

A similar test can be carried out by means of the expansibility and its temperature coefficient in the following way: As a consequence of the formula of Drude and Nernst (8), the quotient

$$[\phi^0(t_1) - \phi^0(t_2)]/[\phi^0(t_2) - \phi^0(t_3)]$$

where ϕ^0 denotes the apparent molal volume at infinite dilution, should be independent of the nature of the electrolyte and equal to a similar quotient in terms of $1/D^2 \cdot (\mathrm{d}D/\mathrm{d}P)$. As discussed later, the value of $\mathrm{d}D/\mathrm{d}P$ can be calculated from the variation of the apparent molal volume with concentration. Using the data of Baxter and Wallace (4) for fifteen alkali halides, we found the same order of magnitude for these quotients. Considering the uncertainty of the data, extremely magnified in the involved calculation, a detailed report of the results is hardly justified.

The sensitivity of these methods of testing is greatly impaired by the fact that they are based upon derivatives of the measured properties. Much more sensitive, and therefore decisive, is a test which compares the two quantities which immediately can be measured: namely, ΔC_p and ΔV. In the following this test is discussed for acetic acid.

A TEST OF BORN'S EQUATION

The harmonic mean ionic radius r of a weak electrolyte can be derived independently and with considerable accuracy from the change of heat capacity, ΔC_p, and the change of volume ΔV, accompanying ionization. If Born's equation is valid within the restrictions discussed in the preceding section, and if the non-electric parts of these two properties are negligible, the two values of r should coincide within the experimental error.

In order to discuss ΔC_p, we start, following the procedure of La Mer and Brescia (21), from Born's equation, a thermodynamic relation, and an empirical formula of Åkerlöf as used by Wyman and Ingalls (40):

$$F_e = \frac{N\epsilon^2}{Dr}; \quad C_p = -T\frac{\partial^2 F}{\partial T^2}; \quad D = ae^{-bT} \tag{1}$$

(F_e = electric part of the free energy; N = Avogadro's constant; ϵ = elementary electric charge; D = dielectric constant of water). The change of heat capacity is represented by

$$\Delta C_p = -\frac{N\epsilon^2 b^2 T}{Dr} \tag{2}$$

or, with the numerical values $N = 6.03 \times 10^{23}$, $\epsilon = 4.796 \times 10^{-10}$, $b = 2.303 \times 0.00198$, and $D = 78.54$ (40),

$$\begin{aligned}\Delta C_p &= -109.5/r \text{ (joules}\cdot\text{degree}^{-1}\cdot\text{mole}^{-1}) \\ &= -26.2/r \text{ (calories}\cdot\text{degree}^{-1}\cdot\text{mole}^{-1}) \quad (25°\text{C.})\end{aligned} \tag{3}$$

r being expressed in Ångström units.

From the variation with temperature of the ionization constant, La Mer and Brescia derive $r = 0.704$, corresponding to $\Delta C_p = -37.2$ calories·degree^{-1}·mole^{-1}.[4] From the same experimental material, Harned and Owen (15) found $\Delta C_p = -41.3$, the difference illustrating the uncertainty involved in the computation of a second derivative.

A reliable value of ΔC_p can be calculated with the aid of the heat capacities of acetic acid solutions determined by Richards and Gucker (31). Table 1 contains the molality m, the apparent molal heat capacities $\phi(C_p)$ at the temperatures of measurement, the values for 25°C. found by parabolic extrapolation, and the values $\phi_u(C_p)$ of the undissociated acid obtained by adding $-\alpha\Delta C_p$, where the degree of ionization, α, was computed from the ionization constant given by MacInnes (22). The limiting value for infinite dilution $\phi_u^0(C_p) = 174 \pm 4$ joules·degree^{-1}·mole^{-1} was found, the uncertainty being due mainly to the temperature correction.

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[4] La Mer and Brescia give -39.9, which value, however, is not in accord with their other data.

With Rossini's (32) value $\phi_i^0(C_p) = 17$ joules·degree^{-1}·mole^{-1} for the ions $H^+ + Ac^-$, we obtain

$$\begin{aligned}\Delta C_p &= -157 \pm 5 \text{ joules}\cdot\text{degree}^{-1}\cdot\text{mole}^{-1}\\ &= -37.6 \pm 1.2 \text{ calories}\cdot\text{degree}^{-1}\cdot\text{mole}^{-1}\end{aligned} \qquad (4)$$

$r = 0.697 \pm 0.023$, in excellent agreement with La Mer and Brescia.

The application of the electrostriction formula of Drude and Nernst (8)

$$\Delta V = \frac{\partial \Delta F_e}{\partial P} = -\frac{N\epsilon^2}{D^2 r}\cdot\frac{\partial D}{\partial P} \qquad (5)$$

requires the knowledge of the pressure coefficient of the dielectric constant. The direct determinations of this coefficient were discussed in the fourth paper of this series (29). A much more reliable value can be derived from the limiting value $d\phi/dc^{1/2} = k = 1.86$ (25°C.; ϕ, apparent molal volume; c, concentration in moles per liter), meanwhile confirmed by new measurements (30). According to a relation previously derived (27), we obtain (25°C.)

TABLE 1

Apparent molal heat capacities of acetic acid solutions in joules/degree mole

m	$\phi\ (C_p)$			$\phi\ (C_p)$ EXTRAPOLATED 25°C.	$\phi_u\ (C_p)$ 25°C.
	16°C.	18°C.	20°C.		
0.0695	160.1	160.0	161.3	170.9	173.3
0.139	161.9	162.5	164.5	174.3	176.0
0.278	161.5	161.8	163.8	175.2	176.4
0.555	160.20	161.03	162.61	170.3	171.2
1.11	157.42	158.22	159.86	167.6	168.2
2.22	152.37	153.18	154.83	162.8	163.2

$$\frac{1}{D}\cdot\frac{\partial D}{\partial P} = \frac{\beta}{3} + \frac{k}{2 \times 2.303RTh} = (47.7 \pm 0.3) \times 10^{-6} \text{ atm.}^{-1} \qquad (6)$$

with $\beta = 45.0 \times 10^{-6}$ atm.$^{-1}$ (compressibility) and $h = 0.5056$ (coefficient of the Debye–Hückel law). Thus

$$\Delta V = -\frac{8.31 \pm 0.06}{r} \text{ cc. per mole} \qquad (7)$$

To determine with the desired accuracy the volume change of ionization, i.e., of the reaction

$$HAc + NaCl = HCl + NaAc$$

in the limit of infinite dilution, new measurements of the molal volumes of acetic acid, sodium acetate, and hydrochloric acid were required, because the insufficient precision of earlier data in the dilute range caused a large uncertainty of extrapolation. A report of the experimental work will be given in the next papers of this series (30). The molal volume of sodium chloride is well established by the work of Geffcken, Beckmann, and Kruis (10) and of Wirth (39). The values of the apparent or the partial molal volumes at infinite dilution are (in milliliters per mole):

HAc	51.942 ± 0.01	HCl	17.830 ± 0.01
NaCl	16.60 ± 0.03	NaAc	39.244 ± 0.03

so that

$$\Delta V = -11.47 \pm 0.05 \text{ cc. per mole; } r = 0.725 \pm 0.006 \text{ Å}. \quad (8)$$

The two values of r, equation 4 and 8, derived from Born's equation by means of independent experimental data, agree within the limits of error. The main source of uncertainty, the temperature correction applied to the data of Richards and Gucker, is apt to result in too low a value of r. The agreement is, therefore, very satisfactory.

Of course, extensive experimental material will be required before a general conclusion as to the validity of Born's equation within restricted limits can be drawn. The present result appears to justify further experimental work in this direction.

ON THE VALIDITY OF THE LIMITING LAW OF THE MOLAL VOLUME

The discussion presented in the preceding section makes use of the limiting law of the apparent molal volume of electrolytes (27, 28) in a twofold way. First, the extrapolation of the molal volumes to infinite dilution and the uncertainty involved in this extrapolation depend on the validity of this law. Second, the method of calculating[5] the value of dD/dP would be entirely unjustified if the limiting law did not hold true.

It was believed that the experimental material available ten years ago was sufficient to support the limiting law. In addition, some more recent data of high precision reviewed in the fourth paper of this series (29) confirmed this law. Still, some objections were raised meanwhile which made a further investigation necessary, especially since the experimental basis was admittedly small.

Stewart (35, 36) examined the density data given for one hundred and ninety-seven strong electrolytes in the *International Critical Tables* and the Landolt-Börnstein *Tabellen*, and concluded that the limiting law was not valid. In six cases even a decrease of the molal volume with increasing concentration was found, strictly contrary to theory. Stewart's negative result is, in general, a consequence of the use of data obtained for too high concentrations, and in some cases of secondary effects like hydrolysis or incomplete ionization. In

[5] This method has previously been used in unpublished work by Professor M. Randall.

some cases, e.g., potassium chlorate, the negative result was to be explained only by gross mistakes in the data.

Our results (30) for potassium chlorate are represented in figure 1, in which the apparent molal volume is plotted against the square root of the concentration $c^{1/2}$.

Scatchard (34) has pointed out that the square root of the molality should be used rather than $c^{1/2}$ in the limiting law of the activity coefficient. However, for the sake of easier comparison with earlier data, we prefer to keep $c^{1/2}$ until a general revision of the available experimental material has been made.

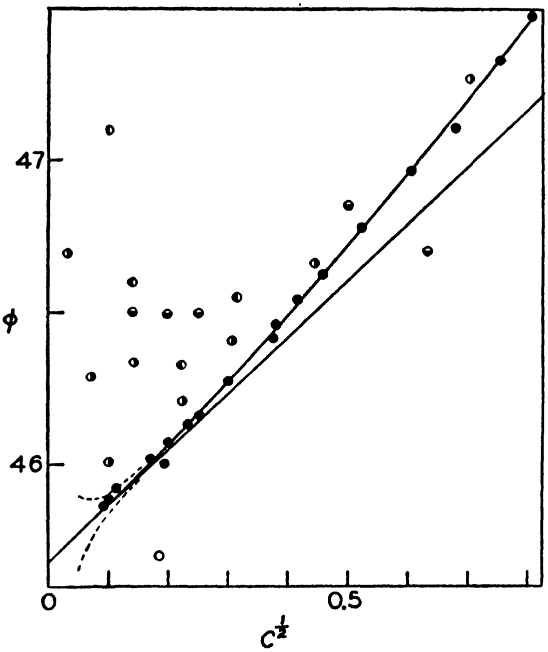

FIG. 1. Apparent molal volumes of potassium chlorate. ○, *International Critical Tables* Vol. III (25°C.); ⊖ Joy and Wolfenden (19) (interpolated to 25°C.); ◐, Jones and Talley (17) (25°C.); ◑, Jones and Ray (18) (25°C.); ●, this paper (24.81°C.).

The curve in figure 1 represents the equation

$$\phi = 45.677 + 1.86c^{1/2} + 0.418c \quad (\text{KClO}_3 \text{ at } 24.81°\text{C.}) \tag{9}$$

the straight line corresponds to the limiting slope 1.86 previously derived. The broken lines indicate the influence of an error of $\pm 3 \times 10^{-7}$ in the density, which corresponds to the sensitivity of our reading device.

The limiting slope is fully confirmed by these data. Our results definitely cannot be represented by a straight line with a different slope, according to Masson's rule rather than to the theoretical limiting law.

The data of some earlier observers (17, 19) have been included in the diagram.

A number of other data are outside the ordinate range. The results of Jones and Ray (18), published after our measurements were finished, agree above $c^{1/2} = 0.25$ within a few hundredths with ours, if the difference of temperature is corrected for.

Only the lowest point of the data given in the *International Critical Tables* falls within the ordinate range of figure 1. Obviously these data, which indicate a negative slope, are grossly erroneous.

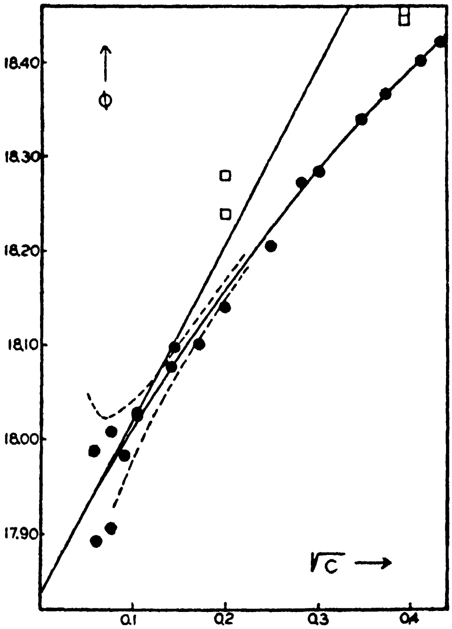

FIG. 2. Apparent molal volumes of hydrochloric acid at 25.00°C. □, Wirth (39); ●, this paper.

Hydrochloric acid offered a serious problem, for the following reasons: Wirth's precise data (39) resulted in a linear relationship of the apparent molal volume with the square root of the concentration, but the coefficient was only one-half of the universal value $k = 1.86$. Thus Wirth's results in this case are in strict disagreement with the theory. As the limiting law has been confirmed so far only for salts, a decisive test of an acid was particularly desirable. In addition, accurate data were required for the test discussed in the preceding section.

Figure 2 represents the lowest points of Wirth and our results, which tend to approach Wirth's data at higher concentrations, except for a small systematic discrepancy. The curve corresponds to the equation

$$\phi = 17.830 + 1.86c^{1/2} - 1.15c \quad \text{(HCl at 25.00°C.)} \qquad (10)$$

The agreement with the predicted limiting slope is perfect.

A RELATIONSHIP INVOLVING THE MOLAL VOLUME AT INFINITE DILUTION

A relationship of some sort between the value ϕ^0 of the apparent or partial molal volume at infinite dilution and the coefficient of the first-power term in equations like 9 or 10 may be expected. This coefficient, which we shall denote by B, represents a measure of the deviations from the limiting law at moderate concentrations and corresponds to a term of the extended Debye–Hückel law. The quantity ϕ^0 may be split into a non-electric or "true" volume and an electrostriction term, as given by the formula of Drude and Nernst. Each of these three terms,—namely, the coefficient B, the "true" volume, and the electrostriction term,—can be represented by means of an "ionic radius." Unfortunately, these ionic radii are little more than arbitrary parameters, so that they must not be identified with one another. Still, they are somehow related to one another, and the same may therefore be expected of ϕ^0 and B.

Indeed, a striking parallelism between ϕ^0 and B has already been mentioned ten years ago (28) in the second paper of this series. This parallelism has been found both by an inspection of the variation with temperature of these two quantities and by a comparison of the values for different alkali halides. Recently Fajans (9) stated a similar relationship between the electric part of ϕ^0 and the individual deviations from the limiting law at moderate concentrations.

The parallelism between ϕ^0 and B explains an empirical relationship found by Stewart (36). It can easily be seen in a purely formal way that Stewart's structural parameter h corresponds to the volume of the solution, and the quantity $\mathrm{d}h/\mathrm{d}m$, therefore, to the partial molal volume or, within Stewart's experimental error, to ϕ^0. Thus the parallelism between $\mathrm{d}h/\mathrm{d}m$ and the deviations of ϕ from the limiting law at moderate concentrations, as found by Stewart, represents just the relationship discussed above.

SUMMARY

Previous tests of Born's equation are discussed, and a restricted range of possible applicability is stated.

A test of Born's equation is based on a comparison of two independent sets of experimental data: namely, the change of heat capacity and the change of volume accompanying ionization. Data available for acetic acid agree with Born's equation within the narrow limits of experimental uncertainty.

The limiting law of the apparent molal volume of electrolytes, used in this test, is fully confirmed by new determinations of the density of dilute solutions of potassium chlorate and hydrochloric acid, contrary to earlier data.

A qualitative relationship between the apparent molal volume of electrolytes at infinite dilution and the deviations of this property from the limiting law at moderate concentrations is discussed.

REFERENCES

(1) Arrhenius, S.: J. Am. Chem. Soc. **34**, 353 (1912).

(2) Askew, F. A., Bullock, E., Smith, H. T., Tinkler, R. K., Gatty, O., and Wolfenden, J. H.: J. Chem. Soc. **1934**, 1368.

(3) Baughan, E. C.: J. Chem. Phys. **7**, 951 (1939).

(4) Baxter, G. P., and Wallace, C. C.: J. Am. Chem. Soc. **38**, 70 (1916).

(5) Bjerrum, N., and Larsson, E.: Z. physik. Chem. **127**, 358 (1927).

(6) Born, M.: Z. Physik **1**, 45 (1920).

(7) Brönsted, J. N., Delbanco, A., and Volquartz, K.: Z. physik. Chem. **A162**, 128 (1932).

(8) Drude, P., and Nernst, W.: Z. physik. Chem. **15**, 79 (1894).

(9) Fajans, K.: J. Chem. Phys. **9**, 283 (1941).

(10) Geffcken, W., Beckmann, Ch., and Kruis, A.: Z. physik. Chem. **B20**, 398 (1933). Kruis, A.: Z. physik. Chem. **B34**, 1 (1936).

(11) Gibbs, J. W.: *Collected Works*, Vol. I, pp. 86–9. Longmans, Green and Co., New York (1928).

(12) Gross, P., and Halpern, O.: Physik. Z. **26**, 403 (1925).

(13) Gurney, R. W.: J. Chem. Phys. **6**, 499 (1938).

(14) Harned, H., and Embree, N. D.: J. Am. Chem. Soc. **57**, 1669 (1935). Harned, H., and Kazanjian, G. L.: J. Am. Chem. Soc. **58**, 1912 (1936).

(15) Harned, H., and Owen, B. B.: Chem. Rev. **25**, 31 (1940).

(16) Ingold, C. K.: J. Chem. Soc. **1931**, 2179.

(17) Jones, G., and Talley, S. K.: J. Am. Chem. Soc. **55**, 624 (1933).

(18) Jones, G., and Ray, W. M.: J. Am. Chem. Soc. **63**, 290 (1941).

(19) Joy, W. E., and Wolfenden, J. H.: Proc. Roy. Soc. (London) **A134**, 419 (1932).

(20) Kritschewsky, I. R.: Acta Physicochim. U.R.S.S. **8**, 181 (1938).

(21) La Mer, V. K., and Brescia, F.: J. Am. Chem. Soc. **62**, 617 (1940).

(22) MacInnes, D. A.: *Electrochemistry*. Reinhold Publishing Corporation, New York (1939).

(23) Malmström, R.: Z. Elektrochem. **11**, 797 (1905).

(24) Murray-Rust, D. M., and Hartley, H.: Proc. Roy. Soc. (London) **A126**, 84 (1929).

(25) Onsager, L.: J. Am. Chem. Soc. **58**, 1486 (1936).

(26) Owen, B. B.: J. Am. Chem. Soc. **55**, 1922 (1933).

(27) Redlich, O., and Rosenfeld, P.: Z. physik. Chem. **A155**, 65 (1931).

(28) Redlich, O., and Rosenfeld, P.: Z. Elektrochem. **37**, 705 (1931).

(29) Redlich, O.: J. Phys. Chem. **44**, 619 (1940).

(30) Redlich, O., Bigeleisen, J., and Nielsen, L. E.: J. Am. Chem. Soc., in press.

(31) Richards, T. W., and Gucker, F. T., Jr.: J. Am. Chem. Soc. **51**, 712 (1929).

(32) Rossini, F. G.: Bur. Standards J. Research **4**, 313 (1930).

(33) Scatchard, G.: J. Am. Chem. Soc. **47**, 2098 (1925).

(34) Scatchard, G.: Chem. Rev. **19**, 309 (1936).

(35) Stewart, G. W.: J. Chem. Phys. **7**, 381 (1939).

(36) Stewart, G. W.: J. Chem. Phys. **7**, 869 (1939).

(37) Webb, T. J.: J. Am. Chem. Soc. **48**, 2589 (1926).

(38) Wegscheider, R.: Monatsh. **23**, 287 (1902).

(39) Wirth, H. E.: J. Am. Chem. Soc. **62**, 1128 (1940).

(40) Wyman, J., and Ingalls, E. N.: J. Am. Chem. Soc. **60**, 1182 (1938).

(41) Wynne-Jones, W. F. K.: Proc. Roy. Soc. (London) **A140**, 440 (1933).

THE APPARENT MOLAL HEAT CAPACITIES AND VOLUMES OF THE AMINO ACIDS AND THEIR UNCHARGED ISOMERS[1,2]

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Studies of the specific heats and densities of aqueous solutions of glycine, glycolamide, *dl*-α-alanine, β-alanine, and lactamide show that the apparent molal heat capacities of these solutes are linear functions of the concentration in dilute solutions, and that the apparent molal volumes are linear functions of the concentration over the whole experimental range. An extension of the Fuoss theory of dipolar solute interaction predicts that these properties of such non-electrolytes in dilute solution should be linear functions of the concentration (instead of the square root of the concentration, as for electrolytes) and allows us to calculate the limiting slopes. These values are within a factor of 10 of the observed values.

The fact that the apparent molal heat capacities and volumes of the amino acids at infinite dilution are appreciably smaller than those of their uncharged isomers has been explained from an electrostatic point of view, based on Kirkwood's treatment of these dipolar solutes. The difference calculated on this basis is about one-third of that observed experimentally, and further refinements in the treatment might give quantitative agreement.

I. INTRODUCTION

Several years ago a systematic study of the heat capacities, heats of dilution, and densities of aqueous solutions of the amino acids and their uncharged isomers was begun in this Laboratory in an effort to determine the effect of the charged groups on these thermodynamic properties of the solutions. Earlier work (9, 10) on the specific heats and densities of aqueous solutions of glycine and glycolamide indicated that the apparent molal heat capacities were linear functions of the molality in the dilute region (up to about 1 *m*), and the apparent molal volumes were linear functions of the molarity throughout the range of concentration from about 0.1 or 0.2 *m* to the nearly saturated solution. The same relationships were recently found (8) for the heat capacities and volumes of *dl*-α-alanine, β-alanine, and lactamide. The experimental data for glycine, glycolamide, α-alanine, β-alanine, and lactamide may be conveniently summarized by means of figures 1 to 4. For a further discussion of the experimental data and techniques the reader is referred to the articles (8, 9, 10) cited above. With the experimental data on these five compounds we are now in a position to test certain theoretical approaches to the study of the non-electrolytic solution.

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[1] Part of this paper was presented at the Symposium on the Thermodynamics of Electrolytic Dissociation, which was held under the auspices of the Division of Physical and Inorganic Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Missouri, April, 1941.

[2] This work was supported by a Grant-in-Aid from the Abbott Fund of Northwestern University.

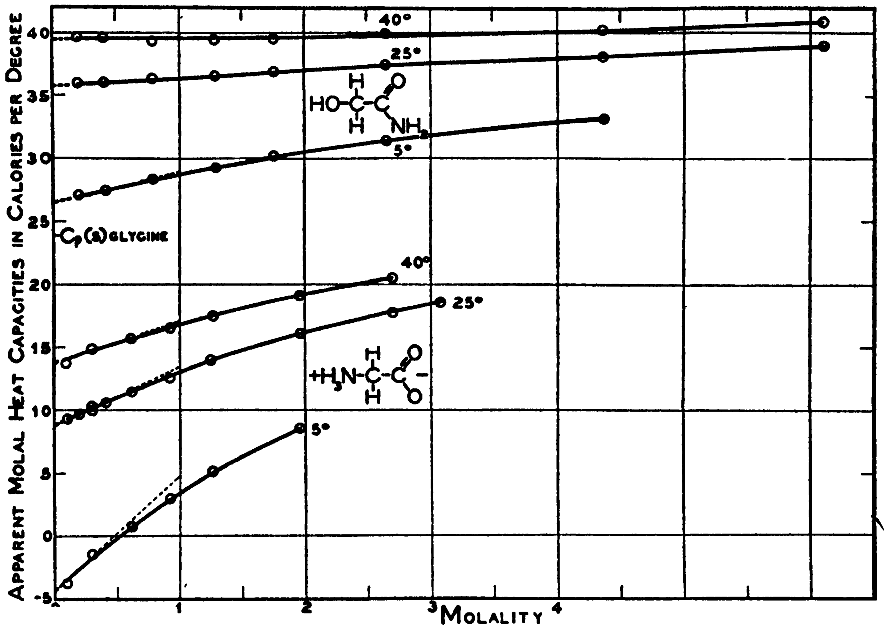

Fig. 1. The apparent molal heat capacities of glycine and glycolamide at 5°, 25°, and 40°C.

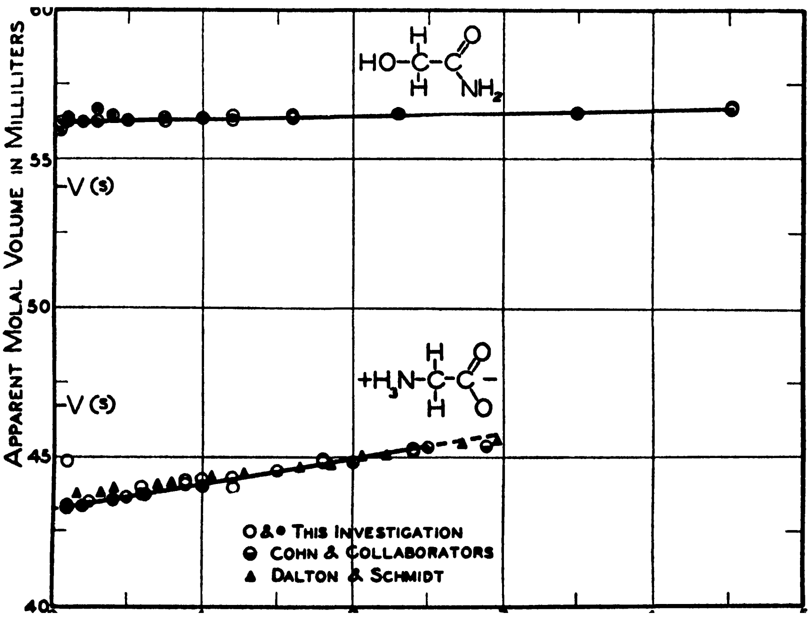

Fig. 2. The apparent molal volumes of glycine and glycolamide at 25°C.

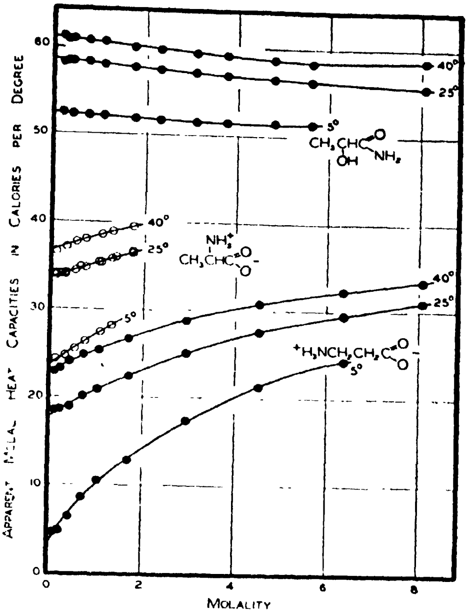

Fig. 3. The apparent molal heat capacities of α-alanine, β-alanine, and lactamide at 5°, 25°, and 40°C.

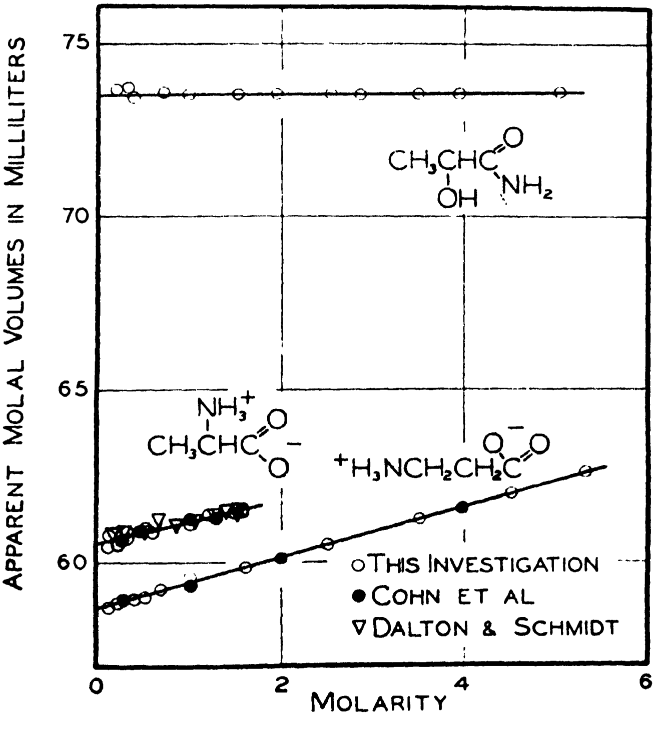

Fig. 4. The apparent molal volumes of α-alanine, β-alanine, and lactamide at 25°C.

II. THEORETICAL INTERPRETATION OF THE RESULTS

An electrostatic treatment of the thermodynamic properties of dipolar solutes may be conveniently divided into two distinct parts. First we shall be interested in the calculation of the limiting slopes of the partial molal heat capacities and volumes, and second, we shall wish to account for the differences between the absolute values of these properties for the amino acids and their corresponding uncharged isomers in the infinitely dilute solution.

As has been pointed out in previous papers (8, 9, 10), we have as yet no theory which will predict quantitatively the behavior of aqueous solutions of dipolar solutes like the amino acids. Fuoss (6) has attempted to describe the thermodynamic properties of such solutions in terms of the mutual interaction of pairs of spherical solute molecules containing central point dipoles. His theory makes use of a parameter:

$$x = \frac{\mu^2}{a^3DkT}$$

where μ is the dipole moment, a the molecular diameter, D the dielectric constant of the solvent, T the absolute temperature, and k the Boltzmann constant. In the present paper this parameter has been modified as suggested by Kirkwood (12) to take into account the discontinuity of the dielectric constant at the surface of the solute molecule. The new parameter y is

$$y = \left[\frac{3D}{2D + D_i}\right]^2 a^3DkT$$

where D_i is the dielectric constant within the spherical cavity containing the dipole.

The senior author, in collaboration with Dr. W. L. Ford (5), has employed this new parameter to calculate the contribution of the dipole-dipole interaction to the apparent molal volumes and heat capacities of dipolar solutes in dilute solutions. If we substitute y in the Fuoss equation for the change in partial molal free energy of the solute due to this interaction, we have simply:

$$\bar{F}_2 - \bar{F}_2^0 = \frac{-RTNJ}{V} \tag{1}$$

where R is the gas constant, N is Avogadro's number, and V is the volume (in liters) of solution containing 1 mole of the solute. J is defined by the equation

$$J = 2a^3y\theta(y) \tag{2}$$

where

$$\theta(y) = \frac{2y}{9}\left(1 + \frac{1}{25}y^2 + \frac{29}{18375}y^4 + \cdots\right) \tag{3}$$

Carrying out the necessary differentiations with respect to temperature, we obtain the following equation for the change in partial molal heat capacity due to dipolar interaction:

$$\bar{C}_{p_2} - \bar{C}^0_{p_2} = RNT^2 \left[2\left(\frac{1}{T} - \alpha\right)\left(\frac{J}{T} - J\alpha\right) + \frac{\partial^2 J}{\partial T^2} - \frac{J}{V}\frac{\partial^2 V}{\partial T^2} \right] \tag{4}$$

where α stands for $\frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P$, the coefficient of expansibility of the solvent. It should be pointed out that this equation differs from that of Fuoss in considering the temperature coefficient of the dielectric constant of the medium. Fuoss' treatment apparently considers the dielectric constant of the medium independent of temperature and hence is not applicable to aqueous solutions.

TABLE 1

Limiting slopes for partial molal heat capacities and volumes

	$\partial\bar{C}_{p_2}/\partial m$ (OBSERVED)	$\partial\bar{C}_{p_2}/\partial m$ (CALCULATED)	$\partial\bar{V}_2/\partial c$ (OBSERVED)	$\partial\bar{V}_2/\partial c$ (CALCULATED
Glycine–glycolamide	7.72	0.55	1.46	0.25
α-Alanine–lactamide	3.92	0.31	1.12	0.16
β-Alanine–lactamide	6.24	0.78	1.40	0.54

Using the values of μ and a from table 2, and the following data, we may evaluate the parameter y for the dipoles under consideration:

$D = 78.54$
$D_i = 1$ (assumed)
$k = 1.37 \times 10^{-16}$ ergs per degree per molecule
$T = 298°$ A.

This leads to a solution of equation 4, and the limiting slopes for glycine, α-alanine, and β-alanine, compared with the observed values, are listed in table 1. The observed values are obtained by subtracting the limiting slopes of the corresponding uncharged isomers from those of the amino acids, i.e., glycolamide from glycine, and lactamide from α- and β-alanine. This gives us the closest approximation for the experimental value of the limiting slope for "pure" dipole–dipole interaction. The agreement between theory and experiment (about a factor of 10) is fair, considering the very simple model and the inaccuracies of the calculation which arise from taking the differences between large numbers.

A consideration of the pressure coefficient of the free-energy equation leads to the corresponding equation for the partial molal volume change due to dipolar interaction:

$$\bar{V}_2 - \bar{V}^0_2 = -\frac{RTN}{V}\left[\frac{\partial J}{\partial y} \cdot \frac{\partial y}{\partial P} + J\beta\right] \tag{5}$$

where $\beta = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T$, the coefficient of compressibility. Table 1 lists the results of the evaluation of the limiting slopes for the partial molal volumes of glycine, α-alanine, and β-alanine at 25°C. The corresponding observed values, obtained in the same manner given above for the heat capacities, are also to be found in the table. Thus the agreement between theoretical and observed values is somewhat better in the case of the volumes than for the heat capacities.

As a qualitative result of the Fuoss theory, the larger the value of the parameter μ^2/a^3, the greater should be the value of the limiting slopes $\partial \bar{C}_{p_2}/\partial m$ and $\partial \bar{V}_2/\partial c$. It is interesting at this point to compare the values of the parameter and limiting slopes for all five amino acids and amides studied to date in this Laboratory. These values are contained in table 2, and graphical comparison is made in figure 5. In order to make the data in table 2 truly comparable, all values of μ, except those of lactamide and glycolamide, were derived from experimental investigations of the dielectric increment (2), and all values of a from the molal volume of the solid substance. The dipole moments of glycolamide and lactamide were roughly estimated from structural considerations.

TABLE 2

Parameters and limiting slopes for five amino acids and amides

	GLYCINE	β-ALANINE	α-ALANINE	GLYCOLAMIDE	LACTAMIDE
$\mu \times 10^{18}$	15.3	18.9	15.3	2	2
$a \times 10^8$	5.28	5.84	5.82	5.54	6.00
$\frac{\mu^2}{a^3} \times 10^{12}$	1.59	1.79	1.19	0.023	0.018
$\frac{\partial \bar{C}_{p_2}}{\partial m}$ (25°C.)	9.16	5.28	2.96	1.44	−0.96
$\frac{\partial \bar{V}_2}{\partial c}$ (25°C.)	1.72	1.43	1.15	0.26	0.034

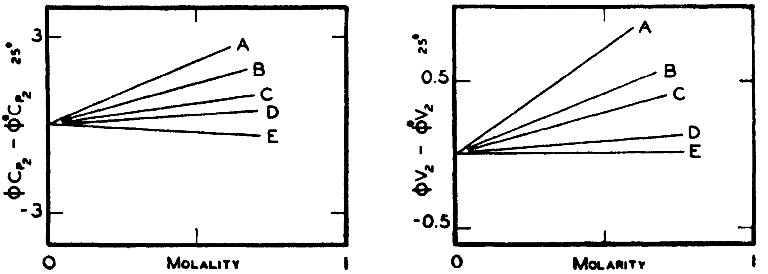

FIG. 5. The limiting slopes of the apparent molal heat capacities and volumes at 25°C. Curve A, glycine; curve B, β-alanine; curve C, α-alanine; curve D, glycolamide; curve E, lactamide.

Rough agreement with the Fuoss theory is shown in all comparisons except that between glycine and β-alanine. According to the theory, the limiting slopes of the partial molal volumes and the partial molal heat capacities should be greater for β-alanine than for glycine, but the opposite is observed. However, this discrepancy may not be significant when one considers the simplicity of the Fuoss model of a point dipole at the center of a sphere, and the sensitivity of the parameter μ^2/a^3 to the choice of values for μ and a. It is obvious that, of the compounds under consideration, the β-alanine molecule would be the least likely to conform to the model of a point dipole at the center of a sphere. A further refinement of the Fuoss model would be to consider the finite distance of separation between the charges of the dipole. Calculations along this line have been started by the senior author, in collaboration with Dr. Hugh B. Pickard, and the results, as yet not complete, indicate a further contribution of about 20 per cent to the thermodynamic properties considered above.

To account for the magnitude of some of the thermodynamic properties of dipolar ions in an infinitely dilute aqueous solution, an electrostatic treatment, employing the Kirkwood (11) formula for the charging energy of an ion, has been adopted. We have accepted the model for an amino acid molecule shown in figure 6 and have calculated the apparent molal heat capacity and volume changes in the reaction

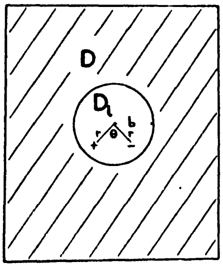

FIG. 6. Model for amino acid molecule

$$\mathrm{HOCHRCONH_2 = H_3^+NCHRCOO^-} \tag{6}$$

For a spherical molecule consisting of an arbitrary charge distribution in a cavity of radius b and dielectric constant D_i, situated in a medium of dielectric constant D and in the absence of electrolyte, the free energy of solvation is given by

$$W_0 = \frac{1}{2}\sum_{n=0}^{\infty}\frac{(n+1)Q_n(D_i - D)}{D_i b^{2n+1}[(n+1)D + nD_i]} \tag{7}$$

where Q_n is

$$Q_n = \sum_{k=1}^{m}\sum_{l=1}^{m} \epsilon_k \epsilon_l r_k^n r_l^n P_n(\cos\theta_{kl}) \tag{8}$$

in which "P_n (cos θ) are the ordinary Legendre functions and θ_{kl} is the angle between r_k and r_l, the lines drawn from the center of the sphere b to the charges

ϵ_k and ϵ_l ." In the reaction represented by equation 6 the change in the charging energy will be given by

$$\Delta W_0 = \frac{1}{2}\sum_{n=1}^{\infty}\frac{(n+1)Q_n(D_i - D)}{D_i b^{2n+1}[(n+1)D + nD_i]} - \frac{1}{2}\sum_{n=1}^{\infty}\frac{(n+1)Q'_n(D_i - D)}{D_i b'^{(2n+1)}[(n+1)D + nD_i]} \tag{9}$$

$$\Delta W_0 = \frac{1}{2}\sum_{n=1}^{\infty}\frac{(n+1)}{D_i}\,\frac{(D_i - D)}{[(n+1)D + nD_i]}\cdot\left[\frac{Q_n}{b^{2n+1}} - \frac{Q'_n}{b'^{(2n+1)}}\right] \tag{10}$$

where the primed symbols refer to the amide. If we let N represent Avogadro's number, then

$$\frac{\Delta\bar{C}^0_{p_2}}{N} = -T\left(\frac{\partial^2 \Delta W_0}{\partial T^2}\right)_P$$

$$= -\frac{T}{2}\frac{\partial^2}{\partial T^2}\sum_{n=1}^{\infty}\frac{(n+1)}{D_i}\,\frac{(D_i - D)}{[(n+1)D + nD_i]}\left[\frac{Q_n}{b^{2n+1}} - \frac{Q'_n}{b'^{(2n+1)}}\right] \tag{11}$$

$$= -\frac{T}{2}\sum_{n=1}^{\infty}\frac{(n+1)}{D_i}\left[\frac{Q_n}{b^{2n+1}} - \frac{Q'_n}{b'^{(2n+1)}}\right]\frac{\partial^2}{\partial T^2}\left[\frac{(D_i - D)}{(n+1)D + nD_i}\right]$$

It follows that

$$\frac{\partial^2}{\partial T^2}\left[\frac{(D_i - D)}{(n+1)D + nD_i}\right]$$

$$= \frac{-(2n+1)D_i[(n+1)D + nD_i]\dfrac{\partial^2 D}{\partial T^2} + 2(2n+1)(n+1)D_i\left(\dfrac{\partial D}{\partial T}\right)^2}{[(n+1)D + nD_i]^3} \tag{12}$$

At this point it is convenient to adopt one of the standard equations for the variation of the dielectric constant of water with temperature. We shall consider first the form used both by Åkerlöf and Short (1) and Wyman and Ingalls (18):

$$\ln D = \alpha - \beta T \tag{13}$$

where α and β are constants; but later we shall also consider the effects of two other types of equations. Using equation 13 and assuming that $D_i = 1$, we may simplify equation 12 to:

$$\frac{\partial^2}{\partial T^2}\left[\frac{(D_i - D)}{(n+1)D + nD_i}\right] = \frac{(2n+1)\beta^2 D[(n+1)D - n]}{[(n+1)D + n]^3} \tag{14}$$

Hence it follows that

$$\frac{\Delta\bar{C}^0_{p_2}}{N} = -\frac{T}{2}\sum_{n=1}^{\infty}(n+1)\left[\frac{Q_n}{b^{2n+1}} - \frac{Q'_n}{b'^{(2n+1)}}\right]\frac{(2n+1)\beta^2 D[(n+1)D - n]}{[(n+1)D + n]^3} \tag{15}$$

It is possible to simplify equation 15 by a consideration of the relative magnitudes of some of its terms. Since, for water, D is of the order of 80, $(n + 1)D \gg n$, and we may neglect n with respect to $(n + 1)D$. Furthermore, since the values of b and b' are roughly equal, whereas $Q_n \gg Q'_n$, we may neglect

$Q'_n/b'^{(2n+1)}$ with respect to $Q_n/b^{(2n+1)}$. In the latter approximation the inclusion of the primed terms would change the value obtained for $\Delta\bar{C}^0_{p_2}$ by less than 2 per cent. Thus we obtain

$$\frac{\Delta\bar{C}^0_{p_2}}{N} = -\frac{T\beta^2}{2D}\sum_{n=1}^{\infty}\frac{(2n+1)}{(n+1)}\frac{Q_n}{b^{2n+1}} \tag{16}$$

According to Cohn (2), Kirkwood has calculated the percentage of the total effect that may be attributed to terms higher than $n = 1$ for the change in the solubility with change in dielectric constant. For glycine the higher multipoles contribute 14 per cent, whereas for α-alanine they are responsible for 33 per cent. Since Kirkwood's calculation is essentially one for the contribution of the higher order multipoles to the charging energy, it is possible to estimate, by a method of comparison of series, the percentage effect in the heat capacities also. Simplifying the equation for the change in solvation energy in the same manner as we have simplified that for the heat-capacity change, we obtain:

TABLE 3

$\Delta\bar{C}^0_{p_2}$ *at 25°C. in calories per mole per degree*

	DIPOLE TERM	ALL TERMS	EXPERIMENTAL
Glycine–glycolamide	−11.3	−13.0	−26.9
α-Alanine–lactamide	−8.3	−11.2	−20.1
β-Alanine–lactamide	−11.0	*	−35.6

* The effect of higher multipoles is unknown, owing to uncertainty in what to take for the shape factor.

$$\Delta W_0 = \frac{1}{2}\sum_{n=1}^{\infty}\frac{Q_n}{b^{2n+1}} \tag{17}$$

If we now consider the ratios of successive terms in the series represented by equations 16 and 17, respectively, we find that the relative contributions of successive terms to the total value of the series is very slightly greater for the heat-capacity series. However, this excess effect is only about 5 per cent of the contribution of the multipoles higher than the dipole, so that the effect on the total heat capacity should be about 15 per cent (14 per cent + 1 per cent) for glycine and about 35 per cent (33 per cent + 2 per cent) for α-alanine.

Table 3 lists the values obtained for $\Delta\bar{C}^0_{p_2}$ by a calculation including only the dipole term of the series and by one including all the terms. These are compared with the experimental values. The total summation is given by the sum of the dipole term plus the per cent of this term (given above for glycine and α-alanine) due to higher multipoles. For the calculation of the theoretical values, the values given in table 4 were adopted for the various constants and parameters.

In the above treatment we have assumed that the variation of the dielectric constant of water with temperature is given by equation 13. A number of other equations, however, have also been found applicable. Thus Everett and Wynne-Jones (4) have called attention to the fact that an equation of the form

$$\log D = A - B \log T \tag{18}$$

represents very closely the data of Kockel (13) and of Lattey, Gatty, and Davies (15). On the other hand, Wyman and Ingalls (18) use a power series to express their results.

For purposes of comparison we have derived expressions for the heat-capacity change for each of the three types of equation for D, and have evaluated these expressions for the difference between glycine and glycolamide. In these calculations we have considered only the first term of the series for $\Delta\bar{C}^0_{p_2}$ and have employed the constants given by the authors for each of the equations. Our calculations, therefore, include not only the effect of the different types of equation but also the discrepancies in the work of the different authors. It should be noted, however, that Wyman and Ingalls (18) fitted their results to an equation of the type of equation 13 and obtained a value of β which does not differ significantly from that of Åkerlöf and Short (1).

TABLE 4

Constants and parameters used in charging energy calculations

	GLYCINE	α-ALANINE	β-ALANINE
μ in Debye units	15	15	19
b in Å	2.64	2.91	2.92

In the Åkerlöf-Short equation, $\beta = 0.00472$ deg.$^{-1}$
At 25°C., $D = 79$.

TABLE 5

Variation in $\Delta\bar{C}^0_{p_2}$ with equation for D; glycine-glycolamide at 25°C.

EQUATION FOR D	$\Delta\bar{C}^0_{p_2}$
$\ln D = \alpha - \beta T$	−11.3
$\log D = A - B \log T$	−4.0
$D = 78.54\ [1 - a(t - 25) + \cdots]$	−9.3

From table 5 it is obvious that the value obtained for $\Delta\bar{C}^0_{p_2}$ is very sensitive to the equation used for the variation of the dielectric constant of water with temperature. However, in all cases, the value obtained for $\Delta\bar{C}^0_{p_2}$ is much smaller than the experimental value, −26.9 calories per degree × mole. It would seem, therefore, either that the model which has been used is too simple to account for the entire heat-capacity change, or, perhaps, that μ^2/b^3 is not truly independent of temperature.

The calculation of the general expression for the change in partial molal volume at infinite dilution accompanying the reaction represented by equation 6 is quite straightforward. The pressure coefficient of equation 10 would be

$$\frac{\Delta\bar{V}_2^0}{N} = \left(\frac{\partial \Delta W_0}{\partial P}\right)_T = \frac{1}{2}\sum_{n=1}^{\infty}\frac{n+1}{D_i}\left[\frac{Q_n}{b^{2n+1}} - \frac{Q'_n}{b'^{(2n+1)}}\right]\frac{\partial}{\partial P}\left[\frac{D_i - D}{(n+1)D + nD_i}\right] \tag{19}$$

which, if we make the usual approximations, can be reduced to

$$\frac{\Delta\bar{V}_2^0}{N} = -\frac{1}{2D^2}\frac{\partial D}{\partial P}\sum_{n=1}^{\infty}\frac{(2n+1)}{(n+1)}\frac{Q_n}{b^{2n+1}} \tag{20}$$

This equation has the same general form as equation 16, for the change in heat capacity. The consideration of the relative contribution of multipoles higher than the dipole, discussed for the heat capacities, is also applicable to the volume changes. Hence we shall calculate $\Delta\bar{V}_2^0$ from the first term of equation 20.

$$\frac{\Delta\bar{V}_2^0}{N} = -\frac{3}{4}\frac{\mu^2}{b^3D^2}\frac{\partial D}{\partial P} \tag{21}$$

Gucker (7) and Redlich (16) have considered the available data for the calculation of the pressure coefficient of the dielectric constant of water. The work of Kyropoulos (14) at 20°C. has been most generally accepted. We shall assume that Kyropoulos' data, which can be expressed by the equation

$$D = 80.79(1 + 59.2 \times 10^{-6}P - 3.28 \times 10^{-9}P^2 - 1.61 \times 10^{-12}P^3 + \cdots) \tag{22}$$

can be applied at 25°C. Since our calculations are quite crude anyway, any change due to the difference between the pressure coefficient of D at 20° and 25°C. is probably insignificant. Utilizing values for the radii obtained from the molal volumes of the solid substances and the pressure coefficient of D obtained from equation 22, we find the values for $\Delta\bar{V}_2^0$ given in table 6.

The agreement between calculated and observed values of $\Delta\bar{V}_2^0$ is about as good as that for $\Delta\bar{C}_{p_2}^0$. The suggestion of Redlich (17) that $\partial D/\partial P$ be determined from the limiting slopes of the partial molal volumes of electrolytes in aqueous solutions was also considered, but his value would not alter our calculated values significantly. However, there is much uncertainty in the value of $\partial D/\partial P$ and hence in the calculated values of $\Delta\bar{V}_2^0$

Table 7 lists the calculated values for b obtained from the first terms of the respective series for the apparent molal heat-capacity and apparent molal volume changes at 25°C. The experimental values for $\Delta\bar{C}_{p_2}^0$ and $\Delta\bar{V}_2^0$ were employed.

Table 7 is a convenient way of comparing the results obtained from different types of measurement, although it minimizes the disagreement between the experimental and calculated values, since any error in the heat capacity or volume change enters as a *cube root* in b. It is apparent, then, that the model used for the calculations listed above is perhaps a good first approximation, but does not account for the entire effect observed experimentally.

Recently, Everett and Coulson (3) have made a more detailed model for ions in aqueous solution, in which they attempt to break up the treatment of the solvent into two different parts. The change in the heat capacity due to the restriction in rotation of the water molecules in the first shell around an ion is calculated by statistical methods, while the contribution of all the other water shells is assumed to be given by the Born charging energy. Such an approach predicts values of the changes in heat capacity upon the ionization of acids which are fairly close to the values experimentally observed.

The above treatment assumes that the ions under consideration are spherically symmetrical, which is not the case for the amino acids. One may attempt, however, to make a very rough application of the method of Everett and Coulson to the amino acids by assuming that the molecule may be represented by a dumbbell, one end containing a sphere of charge $+\epsilon$ and the other a sphere of charge $-\epsilon$. If the radii of these spheres are each about 1 Å., and if each sphere is surrounded by four water molecules in the first shell so that the solvated molecule has a radius of about 4.5 Å., then the predicted value of $\Delta\bar{C}^0_{p_2}$ would be about 22 calories per degree per mole. However, a treatment based on this very crude model disregards the asymmetry of the amino acid molecule and neglects the effect of the field of the positive end of the dipole on the water molecules surrounding the negative end, and *vice versa*. For the present, then, one can only say that this new model can be made to account for practically all of the observed change in heat capacity, and that it predicts the correct sign for the temperature coefficient of the heat capacity.

TABLE 6

Values of $\Delta\bar{V}^0_2$ at infinite dilution at 25°C.

	$\Delta\bar{V}^0_2$ (CALCULATED)	$\Delta\bar{V}^0_2$ (EXPERIMENTAL)
	ml.	*ml.*
Glycine–glycolamide	−4.1	−12.97
α-Alanine–lactamide	−3.1	−12.90
β-Alanine–lactamide	−4.8	−14.79

TABLE 7

Values of b in Å. from consideration of experimental data at 25°C.

	$\Delta\bar{C}^0_{p_2}$	$\Delta\bar{V}^0_2$	FROM SOLID
Glycine	1.97	1.8	2.64
α-Alanine	2.16	1.8	2.91
β-Alanine	2.10	2.0	2.92

III. SUMMARY

The limiting slopes of the partial molal heat capacities and volumes for glycine, α-alanine, and β-alanine have been calculated from the Fuoss theory of dipolar solute interaction. Observed and calculated slopes agree within a factor of about 10 for the heat capacities and slightly better for the volumes.

The differences in the heat capacities and volumes at infinite dilution between these amino acids and their corresponding uncharged isomers have been estimated from an electrostatic standpoint. The calculated values are approximately one-third of the experimental.

Possible refinements in the theoretical treatment have been indicated.

REFERENCES

(1) Åkerlöf, G., and Short, O. A.: J. Am. Chem. Soc. **58,** 1241 (1936).
(2) Cohn, E. J.: Ann. Rev. Biochem. **4,** 93 (1935).
(3) Everett, D. H., and Coulson, C. A.: Trans. Faraday Soc. **36,** 683 (1940).
(4) Everett, D. H., and Wynne-Jones, W. F.: Trans. Faraday Soc. **35,** 1380 (1939).
(5) Ford, W. L.: Ph.D. Dissertation, Northwestern University, 1940.
(6) Fuoss, R. M.: J. Am. Chem. Soc. **58,** 982 (1936).
(7) Gucker, F. T., Jr.: Chem. Rev. **13,** 111 (1933).
(8) Gucker, F. T., Jr., and Allen, T. W.: J. Am. Chem. Soc. **64,** 191 (1942).
(9) Gucker, F. T., Jr., and Ford, W. L.: J. Phys. Chem. **45,** 309 (1941).
(10) Gucker, F. T., Jr., Ford, W. L., and Moser, C. E.: J. Phys. Chem. **43,** 153 (1939).
(11) Kirkwood, J. G.: J. Chem. Phys. **2,** 351 (1934).
(12) Kirkwood, J. G.: Chem. Rev. **19,** 275 (1936).
(13) Kockel, L.: Ann. Physik **87,** 417 (1925).
(14) Kyropoulos, S.: Z. Physik **40,** 507 (1926).
(15) Lattey, R. T., Gatty, O., and Davies, W. G.: Phil. Mag. **12,** 1019 (1931).
(16) Redlich, O.: J. Phys. Chem. **44,** 619 (1940).
(17) Redlich, O., and Bigeleisen, J.: Chem. Rev. **30,** 171 (1942).
(18) Wyman, J., Jr., and Ingalls, E. N.: J. Am. Chem. Soc. **60,** 1182 (1938).

SOME HEATS OF DILUTION AND RELATED THERMAL QUANTITIES OF AQUEOUS CADMIUM CHLORIDE, BROMIDE, AND IODIDE SOLUTIONS[1]

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Measurements of the heats of dilution of aqueous cadmium chloride, bromide, and iodide solutions at 15° and 25°C. are reported. The data are treated to obtain relative partial molal heat contents, heat capacities, and entropies. The results are discussed with reference to the Debye-Hückel limiting law in extremely dilute solutions and in terms of ion association in more concentrated solutions. Comparison is made with similar data obtained from E.M.F. measurements.

I. INTRODUCTION

The heats of dilution of strong electrolytes have been measured precisely in many cases and have afforded significant tests of the limiting law of Debye and Hückel (3, 6, 20). Analytical treatment of the data, particularly by Young and his coworkers (24, 25, 26), has shown excellent agreements with the theory for sixteen salts of the alkali metals of the 1–1 and 1–2 valence types. Young and Seligmann (25) consider this agreement "to be the most impressive quantitative confirmation yet received by the Debye–Hückel limiting law." Similar treatment of the results for the heats of dilution of 2–2 salts (23; 25, footnote 19) has shown deviations from the limiting law to a greater extent than could be accounted for by experimental error. Recently, however, using a slight modification of the treatment of Young, which will be described later in this paper, we have been able to show (18) that there is strong indication that better agreement with the limiting law exists than previously reported for the heats of dilution of these salts. In the cases of the sulfates of calcium, magnesium, copper, zinc, and cadmium, approach to the limiting law first becomes evident at concentrations below 0.001 *m*.

Although values for the relative partial molal heat contents and related quantities have been obtained for a number of weaker electrolytes by measurements with suitable cells, there is a lack of precise calorimetric measurements for these salts. It was with the purpose of extending our knowledge of the thermochemical properties of dilute aqueous solutions of such salts that these measurements were undertaken.

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II. APPARATUS AND METHODS

The apparatus and method for determining heats of dilution have been adequately described by Lange and Robinson and their coworkers (4, 8, 14, 15, 22). The present experiments were performed using a Lange-type adiabatic differential calorimeter in which the temperature was measured by means of two 665 junction iron–constantan thermels connected in parallel. By observing the deflection of a Leeds and Northrup HS galvanometer connected directly to these thermels, a temperature change of 10^{-7} degree could be detected, although, for most measurements, the electrical resistance of the circuit was so arranged as to limit the sensitivity to approximately 1 microdegree per millimeter deflection.

The calorimeter was identical with that used by Wallace and Robinson (22), with the exception of a few minor details.

Because of the susceptibility of iodide ion to atmospheric oxidation, an oxygen-free system was thought desirable. Oxygen-free water was prepared by boiling freshly distilled water and storing it under an atmosphere of commercial tank nitrogen until used. This "oxygen-free" water was used for the preparation of all cadmium iodide solutions. This same water was used as the dilution water in the calorimeter for all the measurements on cadmium iodide. Suitable precautions were taken to prevent the solution of atmospheric oxygen during the process of filling the calorimeter, and the various entrances to the system were blocked by oil traps to maintain the isolation of the calorimeter contents during the subsequent course of the experiment.

Agreement between results of experiments in which atmospheric oxygen was eliminated and those in which no particular precautions were exercised was taken as evidence that chloride and bromide solutions are not appreciably affected by atmospheric oxygen. Hence the experiments on the chloride and bromide solutions were performed without any attempt to exclude oxygen.

After a few experiments were performed using cadmium iodide solutions, it became evident that surface adsorption was making a small but appreciable contribution to the measured heats. Such effects were rendered inappreciable by coating the calorimeter walls with paraffin, and a suitable revision of the previously accumulated data was made.

The right-hand and left-hand pipets have volumes of 6.755 and 6.782 ± 0.004 ml., respectively. The right-hand and left-hand calorimeters contain 1000 and 1004 ± 1 ml., respectively.

The cadmium iodide and cadmium chloride were Mallinckrodt products of analytical reagent grade, and were used without further purification. The cadmium bromide was a Merck C.P. reagent, and was recrystallized before use. Stock solutions of these substances were made, the halogen contents of which were determined to one or two parts per thousand by titration with a standard silver nitrate solution, using dichlorofluorescein as an indicator. Solutions used in the experiments were prepared by dilution of the stock solutions, using calibrated volumetric glassware.

All solutions were made up by volume and then converted to a weight basis with the aid of densities obtained from the *International Critical Tables.*

III. TREATMENT OF RESULTS

A. Experimental heats of dilution

The scheme of calculation to be followed for these salts is, in general, the same as that employed by Wallace and Robinson for sodium sulfate solutions (22).

For each initial concentration of salt two types of dilution were performed. A small volume, about 7 ml., of salt solution was diluted with about a liter of water and the heat effect (q_1) accompanying the dilution was measured. In the second type of dilution the diluent was the dilute solution produced by the first type of dilution. The heat of this second type of dilution may be called q_2. From these data two heats of dilution may be computed:

$$\Delta H_{c_1 \to c_2} = q_1/n \quad \text{and} \quad \Delta H_{c_3 \to c_2} = 0.5017(q_1 - q_2)/n$$

where c_1 is the initial concentration and c_2 and c_3 are the final concentrations after the first and second types of dilution, respectively. The ΔH's are the heats of dilution per mole of salt for the concentration change indicated. n is the number of moles that was actually diluted, and 0.5017 is a numerical factor arising from the particular volumes of pipets and calorimeters used.[3]

$\Delta H_{c_1 \to c_2}$ corresponds to a dilution of about 148-fold and will be designated hereafter as a long chord. $\Delta H_{c_3 \to c_2}$ corresponds to a dilution of about twofold and will be designated as a short chord. These short chords are in the very dilute range where direct chords are difficult, if not impossible, to obtain. They are particularly important for the practical purpose of extrapolation to infinite dilution and for comparison with the limiting law.

To derive the equation as given above, one considers q_2 as consisting of heats of dilution of n moles of solute from c_1 to c_3 and $n(1 - \delta)$ moles of solute from c_2 to c_3. The quantity δ (= volume of pipet ÷ volume of calorimeter) enters as a result of the procedure followed when using a Lange-type calorimeter (14). Before filling the pipet for making the second dilution it must be emptied of its contents,—namely, the solution remaining after the first dilution. This removes $n\delta$ moles of solute, so there remains $n(1 - \delta)$ moles of solute. Then,

$$\begin{aligned} q_2 &= n\Delta H_{c_1 \to c_3} + n(1 - \delta)\Delta H_{c_2 \to c_3} \\ &= n\Delta H_{c_1 \to c_3} - n(1 - \delta)\Delta H_{c_3 \to c_2} \end{aligned}$$

Also

$$\Delta H_{c_1 \to c_2} = \Delta H_{c_1 \to c_3} + \Delta H_{c_3 \to c_2}$$

from which

$$q_2 = n\Delta H_{c_1 \to c_2} - n(2 - \delta)\Delta H_{c_3 \to c_2}$$

[3] This procedure is slightly different from that followed by Wallace and Robinson (22). They calculated $\Delta H_{c_1 \to c_2}$ and $\Delta H_{c_1 \to c_3}$ and then obtained $\Delta H_{c_3 \to c_2}$ as the difference of these two quantities. This cumbersome method of calculation, while being numerically correct, magnifies excessively the transmission of errors in q_1 and q_2 to $\Delta H_{c_3 \to c_2}$.

TABLE 1

Experimental heats of dilution

INITIAL CONCENTRATION m_i	FINAL CONCENTRATION m_f	AT 25°C.		AT 15°C.	
		q	ΔH	q	ΔH
Cadmium chloride					
moles per kilogram of water		*calories*	*calories per mole of salt*	*calories*	*calories per mole of salt*
0.2005	0.001348	−1.236 ± 0.006	−914 ± 4 (2)*	−0.825 ± 0.010	−610 ± 7 (4)*
0.1005	0.0006768	−0.551 ± 0.002	−812 ± 3 (3)	−0.3904 ± 0.0017	−575 ± 3 (2)
0.05033	0.0003397	−0.2524 ± 0.0008	−741 ± 2 (4)	−0.1766 ± 0.0023	−518 ± 7 (2)
0.02525	0.0001703	−0.1132 ± 0.0021	−662 ± 12 (3)	−0.0748 ± 0.0011	−438 ± 6 (2)
0.01265	0.0000855	−0.0456 ± 0.0003	−532 ± 4 (4)	−0.0294 ± 0.0003	−343 ± 3 (2)
0.002688	0.001348	−0.235 ± 0.012	−88 ± 4 (2)	−0.147 ± 0.000	−55 ± 0 (3)
0.001348	0.0006768	−0.078 ± 0.001	−58 ± 1 (2)	−0.0594 ± 0.0005	−44 ± 0.4 (2)
0.0006768	0.0003397	−0.0301 ± 0.0008	−44 ± 1 (4)	−0.0226 ± 0.0006	−33 ± 0.9 (2)
0.0003397	0.0001703	−0.0073 ± 0.0012	−21 ± 3 (3)	−0.0062 ± 0.0002	−18 ± 0.6 (2)
0.0001703	0.0000855	−0.0037 ± 0.0006	−22 ± 4 (4)	−0.0006 ± 0.0002	−2.5 ± 0.8 (2)
Cadmium bromide					
0.7916	0.005363	−0.166 ± 0.004	−30.9 ± 0.7 (3)		
0.4012	0.002690	−0.009 ± 0.003	−3.3 ± 1.1 (2)	+1.242 ± 0.008	+461 ± 3 (2)
0.2009	0.001350	+0.0753 ± 0.0000	+55.7 ± 0.0 (2)	0.642 ± 0.006	475 ± 4 (2)
0.1006	0.0006775	0.0736 ± 0.0004	108.4 ± 0.6 (3)	0.3160 ± 0.0002	466 ± 0 (2)
0.05043	0.0003400	0.0449 ± 0.0003	131.8 ± 0.6 (3)	0.1523 ± 0.0008	477 ± 2 (2)
0.02527	0.0001705	0.0230 ± 0.0001	134.6 ± 0.6 (2)	0.0686 ± 0.0003	402 ± 2 (2)
0.01266	0.0000856	0.00943 ± 0.00006	110.0 ± 0.7 (2)	0.0286 ± 0.0002	333 ± 2 (2)
0.01069	0.005363	0.306 ± 0.008	28.6 ± 0.7 (3)		
0.005363	0.002690	0.1532 ± 0.0020	28.5 ± 0.4 (2)	0.414 ± 0.002	77.1 ± 0.4 (2)
0.002690	0.001350	0.0634 ± 0.0008	23.5 ± 0.3 (2)	0.181 ± 0.003	67.3 ± 1.1 (2)
0.001350	0.0006775	0.0186 ± 0.0003	13.7 ± 0.2 (2)	0.0607 ± 0.0012	44.8 ± 0.9 (2)
0.0006775	0.0003400	0.0031 ± 0.0003	4.6 ± 0.5 (3)	0.0169 ± 0.0002	24.9 ± 0.3 (2)
0.0003400	0.0001705	0.0011 ± 0.0003	3.2 ± 0.9 (2)	0.0062 ± 0.0002	18.2 ± 0.6 (2)
0.0001705	0.0000856	0.00028 ± 0.00004	1.6 ± 0.2 (2)	0.0007 ± 0.0000	4.1 ± 0.0 (2)

Cadmium iodide					
0.1008	0.0006775	1.517 ± 0.002	2235 ± 3 (2)	1.957 ± 0.004	2883 ± 6 (3)
0.05043	0.0003400	0.7112 ± 0.0002	2088 ± 1 (2)	0.919 ± 0.003	2697 ± 9 (2)
0.02530	0.0001705	0.3056 ± 0.0015	1789 ± 9 (6)	0.4008 ± 0.0014	2345 ± 8 (2)
0.01268	0.00008557	0.1286 ± 0.0008	1500 ± 9 (4)	0.1675 ± 0.0005	1954 ± 6 (4)
0.006356	0.00004264	0.0513 ± 0.0003	1192 ± 6 (4)	0.0699 ± 0.0002	1626 ± 5 (2)
0.003190	0.00002155	0.0206 ± 0.0003	954 ± 7 (4)	0.0264 ± 0.0005	1223 ± 22 (2)
0.001350	0.0006775	0.296 ± 0.007	216 ± 5 (2)	0.398 ± 0.003	295 ± 4 (2)
0.0006775	0.0003400	0.1105 ± 0.0001	163 ± 0 (2)	0.147 ± 0.001	217 ± 1 (2)
0.0003400	0.0001705	0.0352 ± 0.0011	104 ± 3 (6)	0.0476 ± 0.0007	139 ± 2 (2)
0.0001705	0.00008557	0.0092 ± 0.0004	54 ± 2 (4)	0.0152 ± 0.0008	89 ± 5 (2)
0.00008557	0.00004264	0.00265 ± 0.00005	31 ± 1 (4)	0.0050 ± 0.0002	58 ± 2 (2)
0.00004264	0.00002155	0.0014 ± 0.0002	33 ± 5 (4)	0.0008 ± 0.0001	19 ± 2 (2)

* The figures in parentheses indicate the number of runs.

but

$$n\Delta H_{c_1\to c_2} = q_1$$

so

$$\Delta H_{c_3\to c_2} = (q_1 - q_2)/n(2 - \delta)$$

which, after substituting the proper numerical value for δ, gives

$$\Delta H_{c_3\to c_2} = 0.5017(q_1 - q_2)/n$$

In table 1 the data leading to the calculation of the heats of dilution are summarized. The first two columns give the initial and final concentrations of a dilution, respectively. The remainder of the table gives the experimental heat effects and heats of dilution per mole of salt at the two temperatures studied.

Data for the calculation of the short chords have been entered in table 1 just as if they were performed directly. For the short chords c_3 and c_2 are given in the columns headed m_1 and m_2, respectively, while $q_1 - q_2$ has been tabulated under the column headed q.

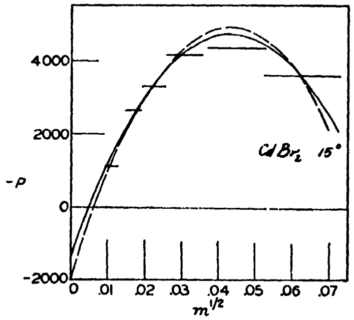

FIG. 1. Plot of the short chords (average value of $d\Phi L_2/dm^{1/2}$) for aqueous cadmium bromide solutions at 15°C. as a function of the square root of the concentration. Solid line calculated from equation 1; broken line calculated from equation 6.

B. The extrapolation

For extrapolation to infinite dilution from the lowest measured final concentration, we have used a modification of the method of Young (24, 25). As an example we use the data for cadmium bromide at 15°C. Figure 1 shows a plot of the short chords derived from the heats of dilution in the very dilute concentration range, obtained in the manner just described. Each of these chords represents the average value of the derivative of the apparent molal heat content with respect to the square root of the concentration in the small concentration range defined by the length of the chords. A smooth curve through these chords may be determined by a graphical or analytical procedure. The intercept of this curve on the ordinate axis gives the experimental limiting slope, and the area under the curve to a concentration m gives the relative apparent molal heat content, or its negative the integral heat of dilution at that concentration.

The heats of dilution of cadmium bromide at 15°C. were of such a sign and magnitude as to be in a range wherein the Lange-type calorimeter operates most successfully. For this reason these data are particularly precise (compared to the other five cases) and hence were chosen for use in the analytical treatment. For representation of these data a quadratic equation in $m^{1/2}$ was chosen, and the constants were determined by the method of least squares. The equation so obtained is

$$S = 1367 - 280{,}490m^{1/2} + 3{,}209{,}580m \tag{1}$$

where $S = d\Phi L_2/dm^{1/2}$.

From equation 1 the limiting slope for cadmium bromide at 15°C. is seen to be 1367. It is obvious that, because of the great curvature exhibited by the plot in figure 1, the limiting slope as evaluated cannot be of very great precision. An estimate of the precision of the limiting slope can be made fairly rigorously by means of the familiar equation for the propogation of precision indices (17). If we have

$$U = U(x_1, x_2, x_3 \cdots x_n) \tag{2}$$

where x_1, x_2, x_3, etc. are measured quantities having probable errors p_{x_1}, p_{x_2}, p_{x_3}, etc., then

$$p_U = \sqrt{\sum_i p_{x_i}^2 (\partial U/\partial x_i)^2} \tag{3}$$

where p_U is the probable error of the derived quantity U.

In the application of the method of least squares the constants which are being sought are expressible as explicit functions of the measured quantities. In this particular case

$$S^0 = S^0(\Delta H_1, \Delta H_2 \cdots \Delta H_n, m_1, m_2 \cdots m_n) \tag{4}$$

where S^0 is the limiting slope, ΔH_1, ΔH_2, etc. are the various measured heats of dilution, and m_1, m_2, etc. are the concentrations. Equation 3 may be used to evaluate the probable error of S^0 in terms of the probable errors in the heats and concentrations. Making the reasonable assumption that the probable errors of the concentrations are negligibly small compared to errors in the heats of dilution,

$$p_{S^0} = \sqrt{\sum_i p_{\Delta H_i}(\partial S^0/\partial \Delta H_i)^2} \tag{5}$$

With the aid of probable errors listed in column 6 of table 1, p_{S^0} is found to be ±1020 or $S^0 = 1367 \pm 1020$. The Debye-Hückel limiting slope for a 2-1 salt at 15°C. is 2044. Hence, the data presented in this paper seem to indicate that cadmium bromide *does* obey the limiting law within the limit of error of the experiments, although such obedience is not apparent at concentrations larger than 0.0001 m.

It is a matter of some interest to derive an equation of the same type as equation 1, using the theoretical value of S^0. Such a treatment gives

$$S = 2044 - 325{,}340m^{1/2} + 3{,}775{,}800m \tag{6}$$

Equations 1 and 6 when integrated with respect to $m^{1/2}$ give, respectively,

$$\Phi L_2 = 1367m^{1/2} - 140{,}250m + 1{,}069{,}870m^{3/2} \tag{7}$$

$$\Phi L_2 = 2044m^{1/2} - 162{,}670m + 1{,}258{,}600m^{3/2} \tag{8}$$

Values for ΦL_2 computed from these equations may be used to evaluate heats of dilution for the same concentrations as were used experimentally. The results of such calculations are presented in table 2. The deviations listed in columns 6 and 7 of table 2 show that an equation containing the theoretical limiting slope is almost as successful in reproducing the measured heats of dilution as is an equation in which the initial slope determined by the method of least squares is used; particularly is this true for the lowest concentrations studied.

The deviations listed in column 6 of table 2 are seen to be for most cases larger than the probable errors assigned to the experimental heats of dilution. This is not surprising, since the latter quantities do not include the systematic errors inherent in such measurements, whereas the deviations do to a certain extent take account of this type of error. Hence, the deviations are more likely to be a proper estimate of the accuracy of these measurements than are the probable errors given.

TABLE 2

Comparison of measured and calculated heats of dilution of aqueous solutions of cadmium bromide at 15°C.

m_i	m_f	ΔH			DEVIATIONS ($\Delta H_{\text{calcd.}} - \Delta H_{\text{exptl.}}$)	
		Experimental	Equation 7	Equation 8	Equation 7	Equation 8
moles of salt per kilogram of water		*calories per mole of salt*			*calories per mole of salt*	
0.005363	0.002690	77.1 ± 0.4	74.8	72.2	−2.3	−4.9
0.002690	0.001350	67.3 ± 1.1	71.2	74.1	+3.9	+6.8
0.001350	0.0006775	44.8 ± 0.9	45.5	47.3	+0.7	+2.5
0.0006775	0.0003400	24.9 ± 0.3	24.7	25.1	−0.2	+0.2
0.0003400	0.0001705	18.2 ± 0.6	12.2	11.5	−6.0	−6.7
0.0001705	0.0000856	4.1 ± 0.0	5.0	4.2	+0.9	+0.1

It might be pointed out that in the treatment given above no assumptions as to incomplete dissociation are involved. The E.M.F. data for these salts have customarily been extrapolated to infinite dilution with the aid of the Gronwall, La Mer, and Sandved (7) extension of the Debye–Hückel theory or by assumption of some plausible association equilibrium.

Qualitatively, all three salts at both temperatures indicate an approach to the limiting law similar to that revealed in figure 1; the precision for the other cases seemed insufficiently high to justify an attempt at quantitative comparison with theory.

Equation 7 may be used to compute values for the relative apparent molal heat contents of cadmium bromide solutions at 15°C. for $m \leq 0.005$. For the other five cases included in this investigation, graphical extrapolations of the short chords were carried out, and estimates of the uncertainties introduced into the ΦL_2 values by the extrapolations were made from a comparison of the ΦL_2's so obtained and those yielded by an extrapolation to the theoretical limiting slope. ΦL_2 values for the higher concentrations were obtained by addition of the measured

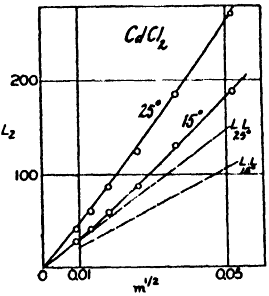

FIG. 2

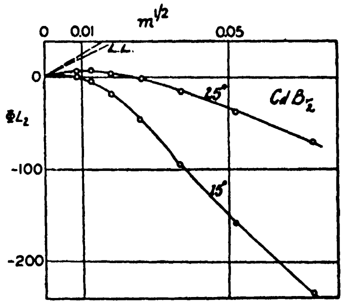

FIG. 3

FIG. 2. ΦL_2 (calories per mole of cadmium chloride) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium chloride solutions at extreme dilutions. Dashed lines represent the Debye-Hückel limiting law for the two temperatures.

FIG. 3. ΦL_2 (calories per mole of cadmium bromide) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium bromide solutions at extreme dilutions. Dashed lines represent the Debye-Hückel limiting law for the two temperatures.

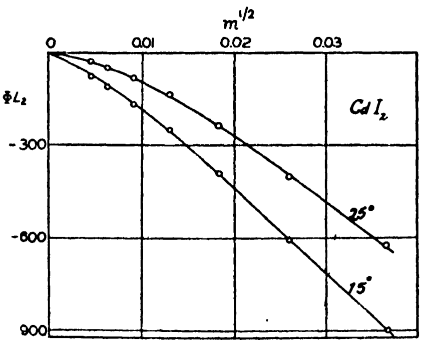

FIG. 4

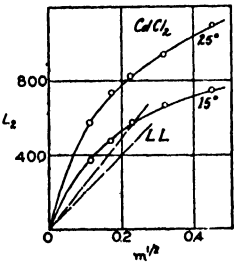

FIG. 5

FIG. 4. ΦL_2 (calories per mole of cadmium iodide) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium iodide solutions at extreme dilutions.

FIG. 5. ΦL_2 (calories per mole of cadmium chloride) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium chloride solutions. Dashed lines represent the Debye-Hückel limiting law for the two temperatures.

heats of dilution to the ΦL_2 values for the various final concentrations. The resulting ΦL_2's are represented graphically in figures 2, 3, 4, 5, 6, and 7.

Values for the relative apparent molal heat contents at several rounded concentrations are displayed in table 3. We estimate the uncertainty of the calorimetric ΦL_2's to be for cadmium chloride 10 calories per mole, for cadmium bromide 5 calories per mole, and for cadmium iodide 15 calories per mole, of which the greater portion is due to the extrapolation.

C. Evaluation of the relative partial molal heat contents, heat capacities, and entropies

Using mechanically determined slopes obtained from large-scale plots of ΦL_2 *versus* $m^{1/2}$, values for $\overline{L}_2$, the relative partial molal heat content, were calculated by conventional methods (19). These values are compared with the results of E.M.F. studies in figures 8 to 10. Also included in table 3 are values for $\overline{L}_2$ at several rounded concentrations.

Figure 8 shows that the $\overline{L}_2$'s for cadmium chloride (11) are in considerable disagreement below 0.01 *m*. Above this concentration there is a constant difference of approximately 375 calories. The estimated uncertainties in the calorimetric $\overline{L}_2$'s are 15 calories per mole. According to Harned (12, 13) a precision of 30 to 50 calories per mole is about as good as can be expected for the E.M.F. $\overline{L}_2$'s, so that if we accept this as a proper estimate of the reliability of the $\overline{L}_2$'s for cadmium chloride (Harned and Fitzgerald do not give an estimate of the precision of their results), the above-mentioned discrepancies are considerably outside the limit of error of the two methods. Harned (10) has indicated the superiority of the calorimetric method for determining heat contents and has pointed out that if "electromotive forces yield results which agree closely with those determined calorimetrically, we have further evidence of their (the electromotive methods') fundamental validity." For some other cases (22) in which disagreement is similar in nature to the above-mentioned, the constant error has been attributed to difficulties in a proper extrapolation of the E.M.F. data and the behavior at low concentrations thought to imply a concentration region too dilute for proper operation of the cells. It is likely that a similar explanation is appropriate here.

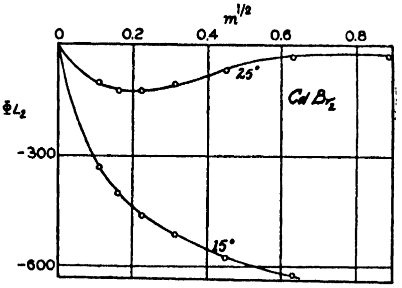

FIG. 6

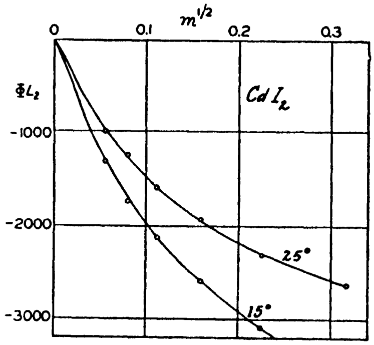

FIG. 7

FIG. 6. ΦL_2 (calories per mole of cadmium bromide) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium bromide solutions.

FIG. 7. ΦL_2 (calories per mole of cadmium iodide) vs. $m^{1/2}$ at 15° and 25°C. for aqueous cadmium iodide solutions.

Figure 9 shows that the $\bar{L}_2$'s for cadmium bromide (1) are, at high concentrations, in agreement within the limit of error of the two methods. However, below 0.01 *m* the discrepancies are larger than could be accounted for on the basis of the uncertainties assigned to the calorimetric $\bar{L}_2$'s (5 calories per mole)

TABLE 3

Relative apparent and relative partial molal heat contents of aqueous cadmium halide solutions

m	ΦL_2		$\bar{L}_2$	
	25°C.	15°C.	25°C.	15°C.
		Cadmium chloride		
moles per kilogram of water	*calories per mole of salt*		*calories per mole of salt*	
0.0001	45	31	67	47
0.0005	105	74	157	112
0.001	152	107	232	162
0.005	411	247	634	362
0.01	563	338	759	472
0.05	833	576	993	702
0.1	948	660	1134	778
0.2	1095	740	1323	860
		Cadmium bromide		
0.0001	6.9	0.3	5.9	−4.0
0.0005	1.4	−32.4	−7.2	−74.3
0.001	−6.8	−68.6	−23.0	−134
0.005	−64.1	−228	−107.8	−343
0.01	−94.5	−302	−131.4	−414
0.05	−138.0	−462	−127.5	−540
0.1	−110.2	−513	−70.1	−591
0.2	−72.8	−569	−8.6	−639
0.4	−35.0	−619	−5.0	−686
0.8	−36.0		−71.2	
		Cadmium iodide		
0.0001	−89	−179	−157	−287
0.0005	−323	−499	−564	−819
0.001	−514	−747	−828	−1148
0.005	−1147	−1556	−1587	−2155
0.01	−1463	−1990	−1941	−2622
0.05	−2305	−3058	−2789	−3700
0.1	−2636	−3480	−3108	−4074

and may indicate a certain amount of unreliability in the behavior of cadmium bromide cells below 0.01 *m*.

Figure 10 shows the $\bar{L}_2$'s for cadmium iodide (2) solutions. Here again, at high concentrations there seems to be a constant error which is larger than could be accounted for by the uncertainties in the calorimetric method (about 20 calories per mole) and suggests the possibility of an improper extrapolation of the E.M.F. data.

Average values for the relative partial molal heat capacities of the solutes, $\bar{C}_{p_2} - \bar{C}^0_{p_2}$, between 15°C. and 25°C., are shown in figure 11 and compared with the results of E.M.F. measurements.

These curves reveal a poorer agreement than has heretofore been obtained for strong electrolytes. We estimate the calorimetric heat capacities to be in error by not more than 3 to 4 calories per degree. Since the electrometric values depend on the second derivative of E.M.F. with temperature, great precision for them is not to be expected. However, the deviations, especially at high concentrations, appear to be greater than could be accounted for by the combined uncertainties of the two determinations and hence imply the existence of some undetermined systematic errors in one or both types of measurements.

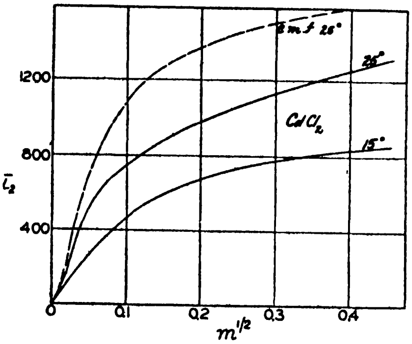

FIG. 8

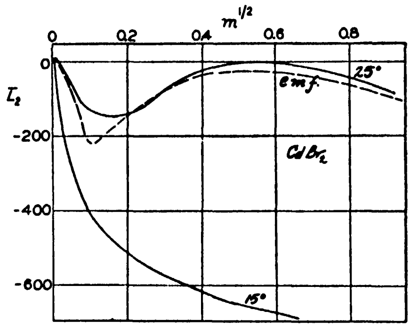

FIG. 9

FIG. 8. $\bar{L}_2$ (calories per mole of cadmium chloride) vs. $m^{1/2}$ at 15° and 25°C. Dashed curve shows E.M.F. results of Harned and Fitzgerald (11) at 25°C.

FIG. 9. $\bar{L}_2$ (calories per mole of cadmium bromide) vs. $m^{1/2}$ at 15° and 25°C. Dashed curve shows the E.M.F. results of Bates (1) at 25°C.

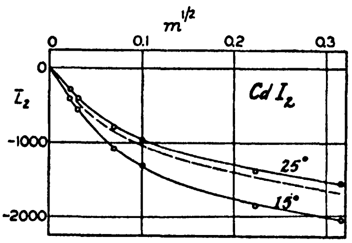

FIG. 10. $\bar{L}_2$ (calories per mole of cadmium iodide) vs. $m^{1/2}$ at 15° and 25°C. Dashed curve shows the E.M.F. results of Bates (2) at 25°C.

We have also computed values for $\bar{S}_2 - \bar{S}^0_2$, the relative partial molal entropies of the solutes, employing activity coefficient values obtained by Harned, Bates, and others (1, 2, 11) from cell measurements. The curves shown in figure 12 represent only the non-ideal part of the entropies; they do not include the ideal entropy of mixing and thus go to a zero value at infinite dilution instead of to infinity. Also included in figure 12 is the non-ideal entropy for calcium chloride, calculated using activity coefficients from the E.M.F. measurements of Scatchard and Tefft (21) and $\bar{L}_2$ values from the heat of dilution data of Lange and Streeck (16). A comparison of calcium chloride and cadmium chloride seems interesting, since the crystal radii of the two cations are nearly the same and therefore, presumably, at least at infinite dilution, their effect on the polarization of surrounding water molecules should be nearly equal.

IV. DISCUSSION OF RESULTS

The abnormalities of solutions of the cadmium halides have long been a subject of interest (see reference 1, footnote 3, and reference 2, footnotes 2 to 6).

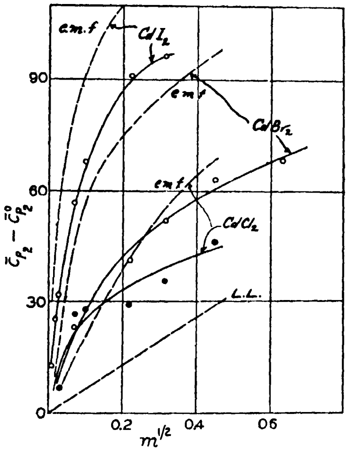

FIG. 11

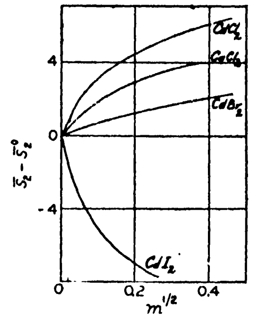

FIG. 12

FIG. 11. $\bar{C}_{p_2} - \bar{C}^0_{p_2}$ (calories per mole per degree) vs. $m^{1/2}$ for aqueous cadmium halide solutions and E.M.F. results (1, 2, 11).

FIG. 12. $\bar{S}_2 - \bar{S}^0_2$ (calories per mole per degree) vs. $m^{1/2}$ for aqueous solutions of cadmium halides and calcium chloride at 25°C.

All indications point to the existence of ion association in these solutions, although the nature and concentrations of the complex species are in general unknown. The thermochemical data presented in this paper seem to confirm the general suspicions harbored concerning these solutions.

Compared with other electrolytes of the same valence type, the ΦL_2 values are unusually negative for cadmium bromide and cadmium iodide and unusually positive for cadmium chloride. Since it has been shown, independent of any assumptions regarding ion association, that there is a fair indication of approach to the limiting law for these three salts, it seems plausible to attribute at least a part of the abnormalities to a heat of dissociation as a concentrated solution is diluted. This would mean that the heat of dissociation is positive for cadmium bromide and particularly so for cadmium iodide and negative for cadmium chloride.

The relative partial molal heat capacities are unusually high for these salts, particularly when compared with the values for a strong electrolyte of the same valence type (9, 22). The shape of the curves is also unusual. After an initial steep rise with $m^{1/2}$, the curves flatten out and become concave toward the $m^{1/2}$ axis. It might be mentioned that the heat capacities of cadmium sulfate (4) show a similar trend. For most strong electrolytes the curves are approximately linear with $m^{1/2}$ up to concentrations of several molal and do not differ greatly from the limiting law. The extra heat capacities of the cadmium halides could be interpreted as due to a heat absorbed by the association change which takes place as a solution is heated. The shape of the curves suggests a resemblance to the trend of $d\alpha/dm$ with m (α is the degree of dissociation), the steep portion of the curve corresponding to the concentration region wherein the degree of dissociation changes most rapidly with m. If this interpretation were correct, it would indicate the largest degree of association for cadmium iodide and the least for cadmium chloride. This is also the order indicated by the apparent heat contents.

Examination of the partial molal entropies of strong electrolyte solutions by Frank and Robinson (5) disclosed that for a given cation the order of $\bar{S}_2 - \bar{S}_2^0$ is $Cl^- > Br^- > I^-$ and all deviations from the limiting law are negative. Assuming complete dissociation for the alkali-metal halides, this order and a part of the deviations were interpreted as an extra orienting effect which an ion can exert on its water envelope. This extra orienting effect is due to the progressive weakening of the *total* water structure produced by ions unable to fit smoothly into this structure and the consequent enhanced ability of the ions to form a well-ordered *envelope* of water. Iodide ion effects the maximum disturbance of the water structure for the alkali halides. The same order is found for the entropies of the cadmium halides.

To consider the possible effects of association we compare the partial molal entropies of cadmium chloride and calcium chloride, the cations of which are of about the same size. Data are not available for a similar comparison of the other halides. From figure 12 we observe that the cadmium chloride entropies are more positive. This extra entropy could be considered, in terms of this discussion, as due principally to extra entropy returned to the water envelope when a pair of ions associates and releases some envelope water.

V. SUMMARY

Heats of dilution of aqueous cadmium chloride, bromide, and iodide have been studied at 15° and 25°C. over a concentration range extending from about 0.0001 m (0.00002 m for cadmium iodide) up to several tenths m (0.1 m for cadmium iodide). Using the chord area method in the extremely dilute range, the measurements have been extrapolated to infinite dilution to obtain values for the relative apparent molal heat contents.

Relative partial molal heat contents, heat capacities, and entropies (non-ideal part) have been evaluated for the concentration range studied.

Qualitatively, approach to the limiting law is indicated for all cases studied.

The one case for which the precision in the extremely dilute range seemed sufficiently high to justify quantitative comparison with theory, *viz.*, that of cadmium bromide at 15°C., gave an experimental limiting slope of 1367 ± 1020, which agrees with the theoretical limiting slope (2044) within the limit of error.

Comparison of thermal properties of the solutions with the corresponding quantities derived from cell measurements reveals deviations which are in most cases larger than could be accounted for by the stated precisions of the measurements. This emphasizes the great difficulties involved in obtaining precise thermal data from cell measurements, particularly for weak electrolytes.

The thermal properties of the cadmium halide solutions are consistent with existing views regarding the presence of particles in the solutions other than the simple anions and cations. Accepting the suggestion that the slow approach of these salts to limiting behavior is to be attributed to incomplete dissociation, the conclusion is reached that dissociation decreases from the chloride to the bromide to the iodide.

REFERENCES

(1) Bates, R. G.: J. Am. Chem. Soc. **61,** 308 (1939).
(2) Bates, R. G.: J. Am. Chem. Soc. **63,** 399 (1941).
(3) Debye, P., and Hückel, E.: Physik. Z. **24,** 185 (1923).
(4) Dunkelberger, T. H., and Robinson, A. L.: J. Am. Chem. Soc. **60,** 1301 (1938).
(5) Frank, H. S., and Robinson, A. L.: J. Chem. Phys. **8,** 933 (1940).
(6) Gatty, O.: Phil. Mag. **11,** 1082 (1931).
(7) Gronwall, T. H., La Mer, V. K., and Sandved, K.: Physik. Z. **29,** 358 (1928).
(8) Gulbransen, E. A., and Robinson, A. L.: J. Am. Chem. Soc. **56,** 2637 (1934).
(9) Hammerschmid, H., and Lange, E.: Z. physik. Chem. **A160,** 445 (1932).
(10) Harned, H. S.: J. Franklin Inst. **225,** 623 (1938).
(11) Harned, H. S., and Fitzgerald, M E.: J. Am. Chem. Soc. **58,** 2624 (1936).
(12) Harned, H. S., and Hecker, J. C.: J. Am. Chem. Soc. **56,** 650 (1934).
(13) Harned, H. S., and Nims, L. F.: J. Am. Chem. Soc. **54,** 423 (1932).
(14) Lange, E., and Monheim, J.: Z. physik. Chem. **A149,** 51 (1930).
(15) Lange, E., and Robinson, A. L.: Chem. Rev. **9,** 89 (1931).
(16) Lange, E., and Streeck, H.: Z. physik. Chem. **A157,** 1 (1931).
(17) Merriman, M.: *Theory of Least Squares.* John Wiley and Sons, Inc., New York (1909).
(18) Robinson, A. L., and Wallace, W. E.: J. Am. Chem. Soc. **63,** 1582 (1941).
(19) Rossini, F. D.: Bur. Standards J. Research **4,** 313 (1930).
(20) Scatchard, G.: J. Am. Chem. Soc. **53,** 2037 (1931).
(21) Scatchard, G., and Tefft, R. F.: J. Am. Chem. Soc. **52,** 2272 (1930).
(22) Wallace, W. E., and Robinson, A. L.: J. Am. Chem. Soc. **63,** 958 (1941).
(23) Young, T. F.: Science **85,** 48 (1937).
(24) Young, T. F., and Groenier, W. L.: J. Am. Chem. Soc. **58,** 187 (1936).
(25) Young, T. F., and Seligmann, P.: J. Am. Chem. Soc. **60,** 2379 (1938).
(26) Young, T. F., and Vogel, O. G.: J. Am. Chem. Soc. **54,** 3030 (1932).

THE CALCULATION OF THE THERMODYNAMIC PROPERTIES AND THE ASSOCIATION OF ELECTROLYTE SOLUTIONS[1, 2, 3]

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A convenient system of representing thermodynamic data for salt solutions is described, in which: (1) the Debye κ is taken proportional to the ionic strength per unit volume of solvent; (2) the Debye limiting law is expressed as an analytical function of the temperature; (3) the mean collision diameter a is taken inversely proportion to μ at constant composition; (4) the function

$$Z = 1 + \kappa a - \frac{1}{1 + \kappa a} - 2 \ln (1 + \kappa a)/(\kappa a)^3$$

is presented in a table of Z vs. $g = \kappa a/(1 + \kappa a)$; (5) the deviations from the Debye-Hückel approximation are treated as an apparent association, with the constant determined to agree with analytical methods for the term proportional to the ionic strength; (6) the calculation of association, either electrostatic or chemical, is made on the assumption that short-range forces are independent of the association; (7) deviations from these relations are expressed graphically, as deviations from a power series in the concentrations.

The methods are illustrated for aqueous solutions of sodium chloride and of sulfuric acid.

The Debye-Hückel limiting law for the electrostatic interaction of ions depends upon the interaction of ions which are very far apart, and so may be related to the charge on the ions and the macroscopic properties of the solvent without any necessity for a detailed model of ion or of solvent. The rest of the Debye-Hückel expression arises from the interactions of molecules which are close together and does depend largely upon the details of the models of the ions and the solvent. The models usually used do not correspond very closely to most of the solutions to which they are applied. The differences between the models and real solutions lead to deviations from the theoretical equations for simple models which begin with the first power of the concentration. We therefore propose that smoothing of the measured properties and calculation of other properties from them be carried out by the use of convenient functions which represent the limiting law exactly and change with the concentration approximately as the functions for simple models, but which do not pretend to

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[2] Contribution No. 479 from the Research Laboratory of Physical Chemistry, Massachusetts Institute of Technology.

[3] Parts of this paper are derived from the thesis presented by Leo F. Epstein to the Faculty of the Graduate School of the Massachusetts Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1939.

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represent these properties exactly beyond the limiting law. Any errors due to these approximations will be grouped with the deviations which arise from the inadequacies of the simple models. This procedure is equivalent to choosing a model which differs from the simple one in a way which leads to only slight differences in behavior, although it differs in a way which cannot be pictured by a simple change in the model.

This proposal must not be interpreted as a recommendation to abandon attempts to determine the properties of more complicated models. It is our belief that further progress in the theory can come only through such attempts. One of the easiest ways to check such theories will be to compare the results of the theories with the experimental results smoothed by our methods.

We express the function κ in terms of moles per unit quantity of solvent rather than moles per unit volume of solution:

$$\kappa = (4\pi\epsilon^2\mathrm{N}^2\rho_0\Sigma_i N_i z_i^2/D_0RTw_0N_0)^{1/2} = (8\pi\epsilon^2\mathrm{N}^2\rho_0\mu/1000D_0RT)^{1/2} \quad (1)$$

in which ϵ is the charge on the proton, N is Avogadro's number, R is the gas constant per mole, T is the absolute temperature, N_i is the number of moles of the i^{th} species and z_i its valence, N_0 is the number of moles of solvent of molecular weight w_0, density ρ_0, and dielectric constant D_0, and μ is the ionic strength in moles per kilogram of solvent, $1000\Sigma_i N_i z_i^2/2w_0N_0$. This procedure has been followed in our laboratory for several years. It has been justified theoretically (25) in that it corresponds to the dielectric constant of the solution being proportional to the concentration of the solvent. To make κ proportional to the concentration in moles per liter of solution, which is the assumption usually made, it is necessary to assume that the dielectric constant is independent of the electrolyte concentration. For most electrolytes the assumption which leads to weight molal concentrations is much more probable than the other one. A still more cogent argument is the pragmatic one that the thermodynamic functions may thus be expressed directly in terms of temperature, pressure, and quantities of the components.

We express the limiting-law ratios of the logarithm of the activity coefficient to the square root of the ionic strength *directly* as a power series of the temperature. Since the smoothing of the density and the dielectric constant are empirical, there is no theoretical argument for or against the expression of $(\rho_0/T^3D_0^3)^{1/2}$ as a simple power series in t. There is a very great practical advantage in a simple analytical expression for the quantity which is the basis of our calculations. Using $T = 273.16$°C., $\mathrm{N} = 6.021 \times 10^{23}$, $\epsilon = 4.805 \times 10^{-10}$ E.S.U., $RT = 2.2711 \times 10^{10}$ ergs (2), the density of water from the *International Critical Tables*, and the dielectric constants of Wyman and Ingalls (31), we obtain for water at 1 atm. pressure

$$\frac{\ln \gamma_k}{z_k^2\sqrt{\mu}} = 1.1254[1 + 0.15471(t/100) + 0.03569(t/100)^2 + 0.02389(t/100)^3] \quad (2)$$

in which γ_k is the activity coefficient of the k^{th} species and t is the Centigrade temperature. We are also interested in

$$\frac{\ln \gamma_k}{z_k^2 \kappa} = 3.472 \times 10^{-8}[1 + 0.10194(t/100) + 0.04269(t/100)^2 + 0.00976(t/100)^3] \qquad (3)$$

and

$$\kappa/\sqrt{\mu} = 0.3241 \times 10^{8}[1 + 0.05217(t/100) = 0.00916(t/100)^2 + 0.00888(t/100)^3] \qquad (4)$$

The maximum deviation of equations 2 and 3 from the value calculated from the density and Wyman's value of the dielectric constant at each temperature is 0.02 per cent, and the average of the absolute values of the deviations is 0.01 per cent. For equation 4 the maximum deviation is less than 0.005 per cent. The equations are consistent to a maximum error of 0.014 per cent, but there is no need for using all three together. If more than equation 2 is used, the more convenient of equations 3 and 4 should be chosen.

The relation of dielectric constant to pressure has not been measured accurately enough to warrant an equation for this relation. Probably the best approximation we can make at present is that, at constant temperature, the dielectric constant is proportional to the density. If this relation is exact, $\kappa/\sqrt{\mu}$ is independent of the pressure, and equations 2 or 3 may be made to apply at various pressures by multiplying the right-hand member of each by ρ_{0p}/ρ_{01}, in which ρ_{0p} is the density of water at the pressure p and ρ_{01} is the density at 1 atm. pressure. Redlich's method of obtaining the coefficients of equations 2 and 3 is discussed later.

We assume that the collision diameter a varies with the temperature as D_0T/ρ_0, so that the product κa is independent of the temperature and the pressure. If the dielectric constant of the solvent is proportional to its density at constant temperature this proposal assumes that a is independent of the pressure; this is strictly consistent with the assumptions made in the derivation of the Debye equations. However, there is a variation of a with the temperature which is not consistent with these assumptions. The assumed decrease in size is only 5 per cent for water from 0° to 100°C. and real ions are neither spherical nor rigid, so that the effective collision diameters of real ions should decrease with the temperature. However, our justification is the pragmatic one that this proposal, together with the first, makes the electrostatic contribution to the free energy a function of the temperature and pressure multiplied by a function of the quantities of the components. The practical advantage is very great. The contribution to any "total" thermodynamic function has the same form as the contribution to the total free energy; the contribution to any "partial" quantity of an electrolyte solute has the same form as the contribution to the partial free energy, or chemical potential, of that solute; and the contribution to any partial quantity of the solvent has the same form as the contribution to the chemical potential of the solvent. If G' be taken as the symbol for the electrostatic contribution to any thermodynamic property, these assumptions make

$$\bar{G}'_k = (g/a)z_k^2x/(1 + x) = (g/a)z_k^2y \qquad (5)$$

in which $x = \kappa a = a'\sqrt{\mu}$, and $y = x/(1 + x)$, and g is defined by this equation. The appropriate value of g for each property may be determined from equation 5 with equation 2 and the corresponding relation to the pressure, since all the thermodynamic functions may be determined from F expressed as a function of T, p, and the N_i's. Then

$$G' = (g/a)\Sigma_i N_i z_i^2(y - Z) \qquad (6)$$

and

$$\bar{G}'_0 = -(g/a)\Sigma_i N_i z_i^2 Z/N_0 \qquad (7)$$

$$\begin{aligned} Z &= [1 + x - 1/(1 + x) - 2\ln(1 + x)]/x^2 \\ &= x/3 - 2x^2/4 + 3x^3/5 - 4x^4/6 + \cdots \qquad (8) \\ &= [1 - y - (1 - y)^3 + 2(1 - y)\ln(1 - y)]/y^2 \\ &= y/3 - y^2/6 - y^3/15 - y^4/30 - \cdots \qquad (9) \end{aligned}$$

We determine Z of equations 6 and 7 from a table of Z vs. y. For small values of x the logarithm cannot be determined accurately enough for the direct determination of Z, but Z may be determined from the power series of equation 8 or that of equation 9. That of equation 9 has the advantage in dilute solutions that the convergence is much more rapid. The use of y as the independent variable has the much more important advantage that y approaches unity as x approaches infinity. A table of Z vs. y with a hundred entries is sufficient to give Z to 0.001 by linear interpolation up to $y = 0.95$. The curve of Z vs. y is shown in figure 1. In table 1 the value of y is the sum of the number at the head of the column and that at the left of the row, the upper entry in each square is the value of Z, and the lower (italicized) entry is the increment of Z for a change of 0.01 in y.

In our early studies of electrolyte solutions we gave a the value zero for all electrolytes and so obtained from the theory only terms in the square root of the ionic strength multiplied by a constant or by integral powers of the concentration of any non-electrolyte solute (23, 24). This method is quite satisfactory for uni-univalent electrolytes at ionic strengths less than unity, and we were able to reproduce our measurements accurately with a five-term series of the form

$$\begin{aligned} (F - F^*)/RT = \Sigma_i N_i[\ln(N_i/w_0N_0) - 1 + Az_i^2\sqrt{\mu}] \\ + \Sigma_{ij}(B_{ij} + C_{ij}\sqrt{\mu})N_iN_j/w_0N_0 \\ + \Sigma_{ijk}(D_{ijk} + E_{ijk}\sqrt{\mu})N_iN_jN_k/(w_0N_0)^2 \qquad (10) \end{aligned}$$

More recently (26) we have taken a' equal to unity for all electrolytes. Guggenheim (8) has suggested that with this value of a', one additional term proportional to m is sufficient to represent the measurements up to an ionic strength of 0.1 M. We find very little advantage in using a' as unity rather than zero up to 1 M, and for higher concentrations there is a great advantage in choosing a' to fit the data.

Guggenheim and Wiseman (8, 9) have also claimed that the term in log γ proportional to m "hardly varies with the temperature," which would require that the corresponding term in the heat of dilution be nearly zero. This is true for hydrochloric acid, as they point out, but it is not true in general. They criticize the freezing-point measurements of Scatchard and Prentiss (22) on aqueous potassium chloride solutions because the value they yield for the coefficient of this term, which Guggenheim calls λ, differs from that obtained from electromotive-force measurements at higher temperatures more than the value yielded by earlier freezing-point measurements. From the heat of dilution measurements of Lange and Leighton (18), we obtain for $d\lambda/dt$ 0.00156 at 12.5°C. and 0.00110 at 25°C. The use of this quantity requires the use of the limiting law, which varies with the temperature like equation 2 rather than the approximate values of Guggenheim. This gives $\lambda = 0.077$ at 20°C. from the measurements of Güntelberg (11) and $\lambda = 0.099$ at 25°C. from the measurements of Shedlovsky and MacInnes (28). At 0°C. the freezing-point measurements of Scatchard and Prentiss yielded 0.056, and earlier freezing-point measurements gave 0.083. The assumption that the value of $d\lambda/dt$ at 12.5°C. is the average from 0° to 20°C. and from 0° to 25°C. gives λ at 0°C. from the electromotive-force measurements as 0.046 and 0.060. The agreement with the freezing-point measurements of Scatchard and Prentiss is excellent.

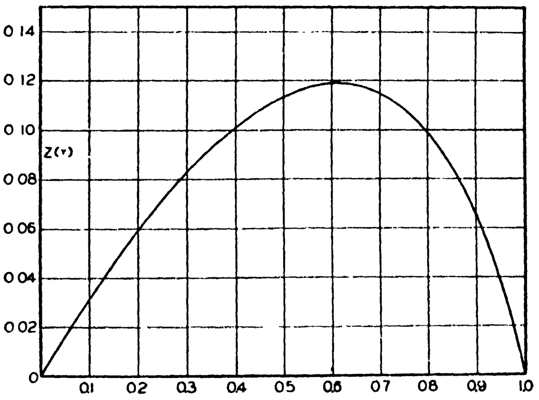

FIG. 1. Debye function Z vs. y

To determine the proper size from measurements of the osmotic coefficient, we plot

$$\Delta\phi = \phi + (A/a')Z_{a'} + b'm \qquad (11)$$

against m, choosing b' so that $\Delta\phi$ is approximately zero for the most concentrated solution. For a single electrolyte $\Sigma_i m_i c_i^2/\Sigma_i m_i = -z_+z_-$, if z_- be taken as negative. The procedure is illustrated for the measurements of the freezing points of aqueous sodium chloride solutions (22) in figure 2, where $\Delta\phi$ is plotted

TABLE 1*

Values of the Debye function, Z

y	0	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	y
0.0	0.00000 *332*	0.03160 *296*	0.05941 *255*	0.08287 *209*	0.10129 *152*	0.11371 *86*	0.11885 *5*	0.11487 *−100*	0.09882 *−244*	0.06537 *−467*	0.0
0.01	0.00332 *328*	0.03456 *292*	0.06196 *251*	0.08496 *203*	0.10281 *147*	0.11457 *80*	0.11890 *−4*	0.11387 *−112*	0.09638 *−261*	0.06070 *−498*	0.01
0.02	0.00660 *325*	0.03748 *288*	0.06447 *246*	0.08699 *197*	0.10428 *141*	0.11537 *72*	0.11886 *−14*	0.11275 *−125*	0.09377 *−280*	0.05572 *−531*	0.02
0.03	0.00985 *321*	0.04036 *284*	0.06693 *242*	0.08896 *193*	0.10569 *134*	0.11609 *64*	0.11872 *−23*	0.11150 *−138*	0.09097 *−299*	0.05041 *−567*	0.03
0.04	0.01306 *318*	0.04320 *281*	0.06935 *237*	0.09089 *187*	0.10703 *128*	0.11673 *56*	0.11849 *−34*	0.11012 *−152*	0.08798 *−320*	0.04474 *−607*	0.04
0.05	0.01621 *314*	0.04601 *276*	0.07172 *233*	0.09276 *182*	0.10831 *122*	0.11729 *48*	0.11815 *−44*	0.10860 *−165*	0.08478 *−341*	0.03867 *−651*	0.05
0.06	0.01938 *311*	0.04877 *272*	0.07405 *228*	0.09458 *176*	0.10953 *115*	0.11777 *40*	0.11771 *−54*	0.10695 *−180*	0.08137 *−363*	0.03216 *−701*	0.06
0.07	0.02249 *307*	0.05149 *268*	0.07633 *223*	0.09631 *171*	0.11068 *108*	0.11817 *31*	0.11717 *−66*	0.10515 *−195*	0.07774 *−387*	0.02515 *−759*	0.07
0.08	0.02556 *304*	0.05417 *264*	0.07856 *218*	0.09805 *165*	0.11176 *101*	0.11848 *23*	0.11651 *−77*	0.10320 *−211*	0.07387 *−412*	0.01756 *−830*	0.08
0.09	0.02860 *300*	0.05681 *260*	0.08074 *213*	0.09970 *159*	0.11277 *94*	0.11871 *14*	0.11575 *−88*	0.10109 *−227*	0.06975 *−438*	0.00926 *−926*	0.09
0.10	0.03160	0.05941	0.08287	0.10129	0.11371	0.11885	0.11487	0.09882	0.06537	0.00000	0.10
y	0.00000	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	y

* y is the sum of the number at the top or bottom of the column and that at the side of the row. Z is the main entry.

The change in Z for a change of 0.01 in y is the lower (italicized) entry.

for a' equal to 1.0, 1.3, and 1.55, with b' equal to 0.0378, 0.0160, and 0.0029. The three curves correspond to the same values of ϕ. The experimental points are shown only above 0.05 M, as those at lower concentrations cluster so closely that they confuse the picture. For a' equal to unity, the curve shows a high maximum. As a' decreases, the maximum becomes lower and shifts to smaller concentrations, and an inflection, which is outside the range of these measurements for $a' = 1$, also shifts to lower concentrations. We try to choose the smallest value of a' which will give neither maximum nor inflection in dilute solutions. Our choice from these measurements is the lower curve with $a' = 1.55$. It is often possible to choose a' so that $\Delta\phi$ may be represented by a quadratic in m, but we have had little success with attempts to reduce it to a linear term as in the Hückel equation. In the present case the smallest deviations from the Hückel equation would occur with a' about 1.3, which corresponds to the middle curve. Since any change in a' will increase the deviations in either dilute or concentrated solutions, both of which are already considerably greater than the scatter of the measurements, no choice of a' will represent these measurements within their apparent accuracy.

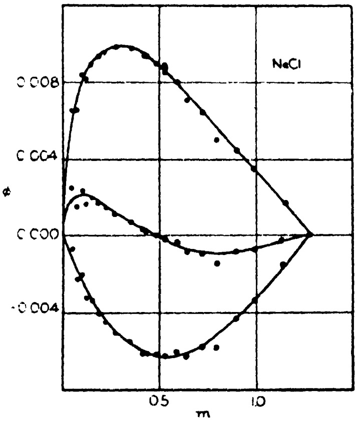

FIG. 2

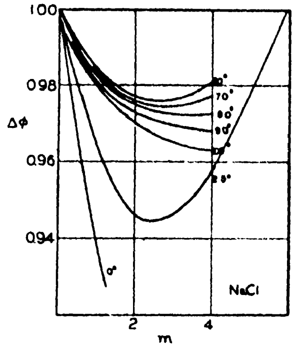

FIG. 3

FIG. 2. Deviation function for osmotic coefficient of sodium chloride for various sizes
FIG. 3. Deviation function for osmotic coefficient of sodium chloride

We illustrate our method of handling the measurements with various properties of aqueous solutions of sodium chloride. In figure 3, the osmotic coefficient is represented by equation 11 with $a' = 1.55$ and $b' = 0.06$. This value of b' is chosen to make $\Delta\phi$ approximately zero for saturated sodium chloride solutions at 25°C. The experimental values are the freezing-point measurements of Scatchard and Prentiss (22), the smoothed curve of Scatchard, Hamer, and Wood (26) at 25°C., and the boiling-point measurements of Smith (29) and of Smith and Hirtle (30) at higher temperatures. It is obvious that $\Delta\phi$ must pass through a maximum between 25° and 60°C.

Figure 4 shows the activity coefficients of sodium chloride from the electromotive-force measurements of Harned and Nims (13), expressed as

$$\Delta \log \gamma = \log \gamma + 0.4343\,[(A/1.55)y_{1.55} - 0.12m] \tag{12}$$

This is the type of equation which we would use to determine the size of a' from measurements which yielded the activity of the electrolyte, but in this case we have used the value of a' already determined, and the $0.12m$ is obtained from the $0.06m$ for the osmotic coefficient.

Figure 5 shows the heat of dilution of sodium chloride from the measurements of Gulbransen and Robinson (10) and of Lipsett, Johnson, and Maas (19), expressed as

$$\Delta(H/m) = (H - H^*)/m - (2RT/1.55)(\mathrm{d}A/\mathrm{d}T)(y_{1.55} - Z_{1.55}) + 140m \tag{13}$$

If the value of a' were not chosen the same as for the measurements of the freezing points, we would use an equation of the type of equation 13 to determine an appropriate value. The term proportional to m is again chosen to give a very small deviation for the most concentrated solution at 25°C. The decrease in $\Delta(H/m)$ with increasing temperature corresponds to the behavior of $\Delta\phi$ and of $\Delta \log \gamma$, for $\Delta(H/m)$ must be zero when the other two pass through maxima.

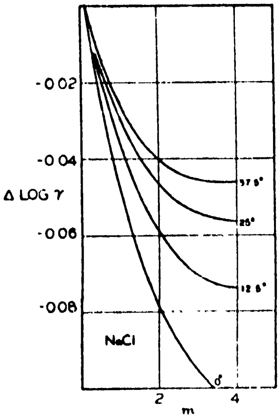

Fig. 4

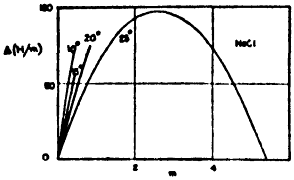

Fig. 5

Fig. 4. Deviation function for logarithm of activity coefficient of sodium chloride

Fig. 5. Deviation function for molal heat of dilution of sodium chloride

Figure 6 shows the apparent molal volumes of aqueous sodium chloride solutions at 25°C. from the measurements of Baxter and Wallace (1), of Geffcken (5), and of Geffcken, Beckmann, and Kruis (6), expressed as

$$\begin{aligned}\Delta\Phi_{v_2} &= \Phi_{v_2} - (2RT\beta A/1.55)(y_{1.55} - Z_{1.55}) - 0.6m \\ &= \Phi_{v_2} - 1.665(y_{1.55} - Z_{1.55}) - 0.6m \end{aligned} \tag{14}$$

in which β is the compressibility. The term $0.6m$ is again chosen to make the deviation approximately zero for the most concentrated solution. The coefficient of the first term corresponds to the assumption that the dielectric constant is proportional to the concentration of water at constant temperature. This leads to a limiting law 7.5 per cent smaller than that determined by Redlich (23) from the apparent volumes of sodium and potassium chlorides and bromides. Dr. Redlich and the authors agree that the available volume measurements are not quite sufficient to furnish an unquestionable decision between the two, and we are not proposing a revision of his value. For our present purpose, however, we consider a value given by an approximate assumption preferable to one obtained from the volume measurements themselves.

In the case of the apparent molal volumes, the form we have chosen for the electrostatic term is not helpful, for the deviation from the limiting law is smaller than that from our expression, and it may be represented more closely by a term linear in *m*.

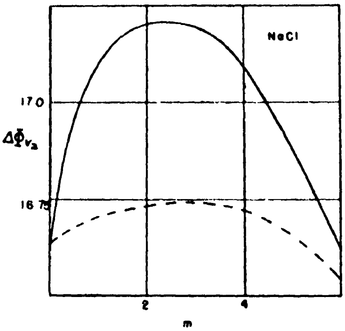

FIG. 6. Deviation function for apparent molal volume of sodium chloride

$$\Delta\Phi_{v_2}' = \Phi_{v_2} - \frac{4}{3}RT\beta A\sqrt{m} - 0.1m = \Phi_{v_2} - 1.72\sqrt{m} - 0.1m \qquad (15)$$

The corresponding function for Redlich's treatment is

$$\Delta\Phi_{v_2}'' = \Phi_{v_2} - 1.86\sqrt{m} \qquad (16)$$

Up to $m = 0.4$, it is a horizontal line at 16.61, which is 0.02 cc. per mole smaller than the intercept of our curves.

If there is association of a considerable fraction of the ions, the deviations corresponding to figures 2 to 6 will be large and the curves will be complex. It is possible to obtain a much more accurate interpolation by taking the association into account. Before discussing the treatment of association, we note that it is not possible to distinguish experimentally between chemical association and that part of the electrostatic interaction which is neglected in the Debye–Hückel approximation, and which is called "electrostatic association" by Bjerrum and the "higher term correction" by La Mer and Gronwall, except in the case that the effect is so large that it leads to an unreasonably small distance *a* when calculated as an electrostatic effect. We believe that the calculation by the law of mass action affords the most satisfactory treatment of the higher term effect. Why is the analytical method of La Mer and Gronwall

(7,17) not entirely satisfactory? Their result is given as the first terms in an infinite power series in $(-z_i z_i \epsilon^2/DkT)$, and partially as the first terms in infinite power series in κa. The computation of any further terms would require considerable labor. Moreover, the result would be uncertain because the conditions of integrability are violated and the value calculated for the work of charging the ions reversibly depends upon the method of adding the charge (15, 20). The conditions of integrability are satisfied for terms in the electrical potential containing the first power of $(-z_i z_i \epsilon^2/DkT)$, which is the Debye-Hückel approximation. They are also satisfied for terms containing the first power of $(\kappa^2 a^2)$, and it is this fact which we utilize. The uncertainty introduced by the violation of these conditions apparently becomes more serious as the exponents of $(-z_i z_i \epsilon^2/DkT)$ and of $(\kappa^2 a^2)$ increase.

The method of Bjerrum (3), on the other hand, suffers from the arbitrary choice of the distance $(-z_i z_i \epsilon^2/2DkT)$ in the calculation of the association constant. Bjerrum's expression of the equilibrium constant of the reaction.

$$\mathrm{A} + \mathrm{B} = \mathrm{AB}$$

which need not be limited to the case in which the valence of the product AB is zero, is

$$K_{\mathrm{AB}} = -\frac{4\pi \mathrm{N}}{1000}\left(\frac{z_A z_B \epsilon^2}{DkT}\right)^3 Q_{\mathrm{AB}} \tag{17}$$

$$Q_{\mathrm{AB}} = \int_2^{b_{\mathrm{AB}}} \frac{e^t}{t^4}\,\mathrm{d}t \tag{18}$$

$$b_{\mathrm{AB}} = -z_{\mathrm{A}} z_{\mathrm{B}} \epsilon^2/DkTa_{\mathrm{AB}} \tag{19}$$

This equation is used only if b_{AB} is more positive than 2. If b_{AB} is more negative than 2, K_{AB} is assumed to be zero.

We choose the value of K_{AB} so as to fit precisely the analytical expression for the term proportional to the ionic strength. This is the logical extension of the method we use for the Debye-Hückel approximation, and it avoids the difficulties of the violation of the conditions of integrability and of the arbitrary distance. To evaluate the constant we use the expression of Kirkwood[5] for the term in the logarithm of the activity coefficient proportional to the ionic strength.

$$K_{\mathrm{AB}} = -\frac{4\pi \mathrm{N}}{1000}\left(\frac{z_{\mathrm{A}} z_{\mathrm{B}} \epsilon^2}{DkT}\right)^3 [B_{\mathrm{AB}} - (B_{\mathrm{AA}} + B_{\mathrm{BB}})/2] \tag{20}$$

$$\begin{aligned} B_{jk} &= \int_0^{b_{jk}} \frac{1}{t^4}(e^t - 1 - t - t^2/2 - t^3/6)\,\mathrm{d}t \\ &= \{b_{jk}^3 E_i(b_{jk}) - \ln b_{jk} - C_0 - e^{b_{jk}}[2 + b_{jk} + b_{jk}^2] \\ &\qquad + [2 + 3b_{jk} + 3b_{jk}^2 + 11b_{jk}^3/6]\}/6b_{jk} \end{aligned} \tag{21}$$

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[5] Private communication from J. G. Kirkwood. We are very grateful to Professor Kirkwood for calculating these terms from the general expression given by him (16) and for computing the B's in table 2. It should be noted, however, that our use of them to calculate the association constant is not the same as his method of calculating that constant.

$C_0 = 0.5772$ (Euler's constant). Some values of B_{jk} and of Q_{jk} are given in table 2.

The constant of equation 20 is finite and positive for all values of b_{AB}. For small values it is proportional to b_{AB}. For larger values it is slightly smaller than the Bjerrum constant. For symmetrical electrolytes, the ratio of our constant to that of Bjerrum is the sum of the third and fifth columns of table 2 divided by the second column. For unsymmetrical electrolytes, which are discussed later, our constant is increased, but only by an amount which is small relative to the difference from Bjerrum's constant. Our constant should be slightly smaller than his to yield the same values of log γ, for our method gives a more negative value for the Debye–Hückel term, since we use the size a and Bjerrum uses $ab/2$. The relative difference between the two constants is very small except in the range of small values of b_{AB} where the Bjerrum treatment is unsatisfactory.

TABLE 2

Some values of Q_{jk} and of B_{jk}

b_{jk}	Q_{jk}	B_{jk}	$-b_{jk}$	$-B_{jk}$
1		0.046	1	0.037
2	0.000	0.105	2	0.069
3	0.325	0.181	3	0.097
4	0.550	0.285	4	0.120
5	0.755	0.414	5	0.141
6	1.041	0.645	6	0.159
7	1.417	0.979	7	0.176
8	1.996	1.525	8	0.192
9	2.950	2.450	9	0.206
10	4.547	4.023	10	0.219
12	13.51	12.84	12	0.242
15	101.8	101.2	15	0.272

If a is independent of the temperature, b_{AB} changes with the temperature as $1/D_0T$; but if a' is independent of the temperature, b_{AB} changes as $(1/D_0T)^{1/2}$. A change in a_{AB} leads to the same relative change in K for small values of b_{AB} and to a more rapid relative change for larger values of b_{AB}. In addition, K is proportional to $(1/D_0T)^3$ from the part outside the integral, so that most of the variation with temperature is independent of the behavior of b_{AB}. We therefore keep a' independent of the temperature, as in the Debye–Hückel approximation.

The treatment of electrolytes of unsymmetrical valence type is complicated by the fact that the Debye–Hückel term is not the only one which depends upon the action at large distances, but there is a second term which results from the fact that the ionic strength effective for a cation depends more upon the anions than upon the cations, and *vice versa*. Formally it arises from the third term in the expansion of the Boltzmann exponential, and it is zero if all the ions have valences z or $-z$. By the Güntelberg–Müller charging process it leads to the following term in $\ln \gamma$:

$$\ln \gamma'' = \frac{1}{6}\left(\frac{\epsilon^2}{DkT}\right)^3 \frac{z_k^3 \kappa^2}{\Sigma_i n_i z_i^2} \Sigma_i n_i z_i^3 \frac{e^{3\kappa a}}{(1 + \kappa a)^3} \int_{t+3\kappa a}^{\infty} \frac{e^{-t}}{t^4} \, dt \tag{22}$$

The Debye charging process leads to a somewhat smaller result, which differs from equation 22 by a power series in κ beginning with κ^2 and $\ln \kappa$ times another power series in κ beginning with κ^3. The contribution to the osmotic coefficient which corresponds to equation 22 contains two similar power series. If we expand and retain only terms in $\kappa^2 \ln \kappa$ and in κ^2 beyond the Debye–Hückel approximation, we obtain by the Kirkwood method and the Debye charging process

$$\begin{aligned}
\ln \gamma_k &= -\frac{\epsilon^2 z_k^2}{2DkT} \frac{\kappa}{1 + \kappa a} - \frac{\epsilon^4 z_k^3}{6(DkT)^2} \frac{\kappa^2}{\Sigma_i n_i z_i^2} \Sigma_i n_i z_i^3 \left[\ln 3\kappa a - C_0 - \frac{1}{12} + 6B_{ik}\right] \\
&= -\frac{\epsilon^2 z_k^2}{2DkT} \frac{\kappa}{1 + \kappa a} \\
&\qquad - \frac{\epsilon^4 z_k^3}{6(DkT)^2} \frac{\kappa^2}{\Sigma_i n_i z_i^2} \Sigma_i n_i z_i^3 \left[\ln 3\kappa a - C_0 - \frac{1}{12} + 3(B_{ii} + B_{kk})\right] \\
&\qquad - \frac{\epsilon^4 z_k^3}{(DkT)^2} \frac{\kappa^2}{\Sigma_i n_i z_i^2} \Sigma_i n_i z_i^3 [B_{ik} - (B_{ii} + B_{kk})/2]
\end{aligned} \tag{23}$$ [6]

The Güntelberg–Müller charging process gives a result which differs only by the omission of the term 1/12 in the first square bracket. Although it arises from the $\kappa^2 \ln \kappa$ term in the electrical potential, the difference is proportional to κ^2. We prefer the Debye charging process, but the difference from the Güntelberg–Müller process is extremely small. This difference is probably an approximate measure of how much the activity coefficient is affected by the violation of the conditions of integrability in this term. The last term of the second form of equation 23 corresponds to the association constant of equation 20, and we treat more concentrated solutions by assuming association and determining the activity coefficients of the ions from the first two terms of this equation with the n_i's and κ determined from the concentrations of the unassociated ions. For symmetrical valence types $n_A z_A^3 = -n_B z_B^3$, so the second term vanishes in the summation.

For unsymmetrical valence types we also take into account higher types of association, although they lead to no effect proportional to the concentration. As an example, for the series of reactions

$$\begin{aligned}
A^+ + B^{---} &= AB^{--} \\
A^+ + AB^{--} &= A_2B^- \\
A^+ + A_2B^- &= A_3B
\end{aligned}$$

---

[6] If B_{ik} of equation 21 is expanded in an infinite power series in b_{ik} and the result is substituted in equation 23, the equation becomes equivalent to that obtained by Gronwall, La Mer, and Sandved (7). Kirkwood's expression in a closed form gives a great advantage over the power series, which converges very slowly.

we calculate a constant for each, using the value of a determined to fit the first. We ignore the very small values of the constant for the association of two anions or two cations, such as

$$\mathrm{B^{----} + AB^{--} = AB_2^{------}}$$

and compensate by replacing $(B_{ii} + B_{kk})$ in the second term by $2B_{ik}$ when i and k are both positive or both negative. If $i = k$, the third term vanishes automatically.

In salt mixtures we determine K for each electrolyte from an appropriate value of a as though that electrolyte were present alone, but use an average value, the same for all the ions, in the first two terms of equation 23. If a_{AA} be taken equal to a_{AB} in the calculation of K_{AB}, the association constant of A and B, but if it be taken equal to $a_{\mathrm{AB'}}$ in the calculation of $K_{\mathrm{AB'}}$, this may lead to different sizes of the ion A. In the second term the value of B_{AA} corresponding to a_{AB} should be used in the coefficient of n_{B}, and the value of B_{AA} corresponding to $a_{\mathrm{AB'}}$, should be used in the coefficient of $n_{\mathrm{B'}}$. In the coefficient of $n_{\mathrm{A'}}$, $(B_{\mathrm{AA}} + B_{\mathrm{A'A'}})/2$ should be replaced by $B_{\mathrm{AA'}}$, which should be calculated from average values of $(a_{\mathrm{AA}} + a_{\mathrm{A'A'}})/2$ weighted according to the contribution of the terms containing these sizes to the chemical potentials.

We calculate the effect of association, either electrostatic or chemical, by assuming that the non-electrostatic interactions do not affect the association, or conversely, that the association does not affect the short-range interactions. One of us (27) has noted that the success of treating reaction kinetics by simple electrostatic theory depends upon the validity of this assumption in many cases. It is probable that many of the failures of the simple theory may be attributed to the failure of this assumption. We have practically no method of measuring the magnitude of these effects, and the assumption that they are zero does lead to great simplification. We thus divide the deviations into two parts: the electrostatic part which is calculated from the concentrations of the species assumed present, and the non-electrostatic part which is calculated from the stoichiometric concentrations of the component ions regardless of any reaction which may have occurred.

We define the apparent constant K'_{AB} by the relations

$$K'_{\mathrm{AB}} = \frac{(\mathrm{AB})}{(\mathrm{A})(\mathrm{B})} = K_{\mathrm{AB}} \frac{\gamma_{\mathrm{A}} \gamma_{\mathrm{B}}}{\gamma_{\mathrm{C}}} \tag{24}$$

If the logarithm of the activity of each ion is divided into an electrostatic part $\ln \gamma_{ke}$, given by the first line of the second form of equation 23 in terms of the species, and a non-electrostatic part given by a series of integral powers of the concentrations as

$$\ln \gamma_k = \ln \gamma_{ke} + 2\Sigma_i \beta_{ki} m_i + 3\Sigma_{ij} \delta_{kij} m_i m_j + \cdots \tag{25}$$

we may state our last assumption as

$$\beta_{k\mathrm{AB}} = \beta_{k\mathrm{A}} + \beta_{k\mathrm{B}}, \qquad \delta_{ki\mathrm{AB}} = \delta_{ki\mathrm{A}} + \delta_{ki\mathrm{B}}, \text{ etc.} \tag{26}$$

which leads to

$$\ln K'_{\mathrm{AB}} = \ln K_{\mathrm{AB}} + \ln \gamma_{\mathrm{A}_e} + \ln \gamma_{\mathrm{B}_e} - \ln K_{\mathrm{AB}_e}$$

$$= \frac{2Az_{\mathrm{A}}z_{\mathrm{B}}\sqrt{\mu}}{1 + a'\sqrt{\mu}} + A^2 z_{\mathrm{A}} z_{\mathrm{B}}(z_{\mathrm{A}} + z_{\mathrm{B}})\Sigma_i m_i z_i^3$$

$$\times \left[\ln 3a'\sqrt{\mu} - C_0 - \frac{1}{12} + 3B_{ii} + \frac{(z_{\mathrm{A}} + z_{\mathrm{B}})^3 B_{\mathrm{AB,AB}} - z_{\mathrm{A}}^3 B_{\mathrm{AA}} - z_{\mathrm{B}}^3 B_{\mathrm{BB}}}{3z_{\mathrm{A}} z_{\mathrm{B}}(z_{\mathrm{A}} + z_{\mathrm{B}})}\right] \quad (27)$$

The second term of equation 27 vanishes if $\Sigma_i m_i z_i^3 = 0$ or if $z_{\mathrm{A}} + z_{\mathrm{B}} = 0$, except for very small terms involving the differences in B's for pairs of ions of the same kind. These terms may well be ignored. Then the second term vanishes if the solution is electrically symmetrical or if the product AB has zero valence.

Computation by these equations is greatly simplified by the simple device of choosing as independent variable the ionic strength rather than the stoichiometric concentration. If we denote by asterisks the stoichiometric concentrations and activity coefficients, and attribute the unstarred coefficients to the species

$$\ln \gamma_k^* = \ln \gamma_k + \ln (m_k/m_k^*) \quad (28)$$

and the mean activity coefficient of the electrolyte is determined as

$$(\Sigma_j \nu_j \ln \gamma_j^*)/(\Sigma_j \nu_j) \quad (29)$$

in which the summation is carried only over the simple ions. The osmotic coefficient is

$$\phi^* = \Sigma_i m_i \left\{(1 - A/a')z_i^2 Z_{a'} - \frac{A^2 z_i^3}{12} \Sigma_j m_j z_j^3 \times [\ln 3a'\sqrt{\mu} + 1 - C_0 - \tfrac{1}{12} + 3(B_{jj} + B_{ii})]\right\}$$

$$\div (\Sigma_j m_j^*) + (\Sigma_{jk} m_j^* m_k^* \beta_{jk})/(\Sigma_j m_j^*) + 2(\Sigma_{jkl} m_j^* m_k^* m_l^* \delta_{jkl})/(\Sigma_{jk} m_j^* m_k^*) \quad (30)$$

in which m_j^* is zero except for the component ions or neutral molecules. The simplest case is that of symmetrical electrolytes with but a single type of association. Fixing the value of the ionic strength fixes the value of K'_{AB} by equation 27 if K_{AB} is known, and it also fixes the concentration of each species of ions, so the concentration of the associated molecules is easily determined from equation 24. Any value of the stoichiometric concentration can be approximated as closely as desired in a very short time.

For unsymmetrical electrolytes it is necessary to choose trial values of as many concentrations as there are associations to be calculated and to find by trial and error the concentrations of the other species. For a single association this is not very difficult, and even for more complicated systems it will usually be possible to select species with concentrations so small that a large relative change will have very little effect on the concentrations of the other species. The complications are increased because the value of K'_{AB}, etc. depends upon the lack of symmetry of the solution as well as upon the ionic strength. This difficulty is not so serious as it appears. Although the number of equations to be satisfied is doubled, the work necessary to satisfy them is not increased so much.

The calculations including association may be illustrated with sulfuric acid. The constant determined by Hamer (12) from measurements in sodium bisulfate–sodium sulfate mixtures is 82 and corresponds to an a of less than 0.7 by equation 20. We may therefore assume that the association is not entirely electrostatic, choose 1.5 as a reasonable value of a', and ignore the association to H_2SO_4. We choose as variable the ionic strength, μ, and the sulfate-ion concentration, which we will call x. The hydrogen-ion concentration is $\mu - x$, the hydrosulfate ion concentration is $\mu - 3x$, the stoichiometric acid concentration is $\mu - 2x$, and

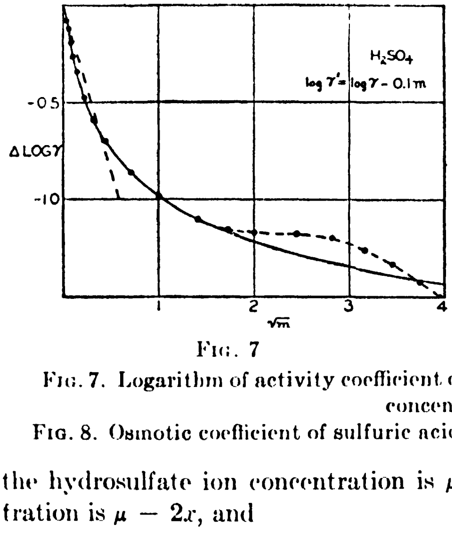

FIG. 7

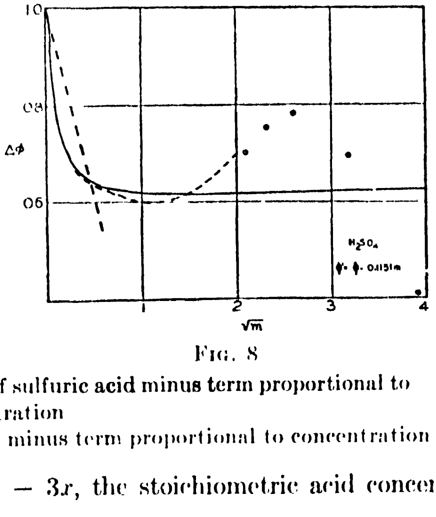

FIG. 8

FIG. 7. Logarithm of activity coefficient of sulfuric acid minus term proportional to concentration

FIG. 8. Osmotic coefficient of sulfuric acid minus term proportional to concentration

$$K'_{HSO_4^-} = (\mu - 3x)/x(\mu - x) \tag{31}$$

The second equation to be satisfied is

$$\log K'_{HSO_4^-} + 1.9124 - 2.036\sqrt{\mu}/(1 + 1.5\sqrt{\mu}) - 16.48\,(\log 4.5\sqrt{\mu} - 0.7887)x \tag{32}$$

For each value of μ, x is varied until equations 31 and 32 yield the same value of $K'_{HSO_4^-}$. Then the activity coefficient and osmotic coefficient are obtained as

$$\log\gamma^* = [2\log(\mu - x) + \log x - \log 4]/3 - \log(\mu - 2x) - 1.018\sqrt{\mu}/(1 + 1.5\sqrt{\mu}) - 5.493\,[\log 4.5\sqrt{\mu} - 0.7887]\,x \tag{33}$$

$$\phi^* = 2/3 + \frac{x - 2.303\,[1.018\,Z_{1.5} + 8.240(\log 4.5\sqrt{\mu} - 0.3544)]\,x^2}{3(\mu - 2x)} \tag{34}$$

The full curve in figure 7 represents equation 33. The broken line, with filled circles to show its course where the two curves overlap, represents the measurements of Harned and Hamer (14) minus $0.1m$. Up to 2 M the agreement is well within the thickness of the lines. The scale of this figure is, of course, much smaller than those in the preceding figures. The full line in figure 8 is the osmotic coefficient calculated from equation 23. The broken line is the smoothed ϕ of Scatchard, Hamer, and Wood (26) minus $0.11513m$, and the circles represent ϕ minus $0.11513m$ from the vapor-pressure measurements of Collins (4) for solutions more concentrated than 4 M. The differences between the calculated and measured values are apparent at lower concentrations than in the preceding figure, but they are small in dilute solutions. In spite of the complications due to the unsymmetrical solution effect represented in equation 22 and to the apparent association, the method works very well up to high concentrations. This application to sulfuric acid includes all the proposals that we have made in this paper.

REFERENCES

(1) Baxter, G. P., and Wallace, C. C.: J. Am. Chem. Soc. **38,** 70 (1916).
(2) Birge, R. T.: Phys. Rev. Suppl. **1,** 1 (1929).
(3) Bjerrum, N.: Kgl. Danske Videnskab. Selskab **7,** 9 (1926).
(4) Collins, E. M.: J. Phys. Chem. **37,** 1911 (1933).
(5) Geffcken, W : Z. physik. Chem. **A155,** 1 (1931).
(6) Geffcken, W., Beckmann, C., and Kruis, A.: Z. physik. Chem. **B20,** 398 (1933).
(7) Gronwall, T. H., La Mer, V. K., and Sandved, K.: Physik. Z. **29,** 358 (1928).
(8) Guggenheim, E. A.: Phil. Mag. [7] **19,** 588 (1935).
(9) Guggenheim, E A., and Wiseman, L. A.: Phil. Mag. [7] **25,** 45 (1938).
(10) Gulbransen, E. A., and Robinson, A. L.: J. Am. Chem. Soc. **56,** 2637 (1934).
(11) Güntelberg, E.: Z. physik. Chem. **123,** 199 (1926).
(12) Hamer, W. J.: J. Am. Chem. Soc. **56,** 860 (1934).
(13) Harned, H. S., and Nims, L. F.: J. Am. Chem. Soc. **54,** 423 (1932).
(14) Harned, H. S., and Hamer, W. J.: J. Am. Chem. Soc. **57,** 27 (1935).
(15) Kirkwood, J. G.: J. Chem. Phys. **2,** 767 (1934).
(16) Kirkwood, J. G.: Chem. Rev. **19,** 275 (1936).
(17) La Mer, V. K., Gronwall, T. H., and Greiff, L. J.: J. Phys Chem. **35,** 2245 (1931).
(18) Lange, E., and Leighton, P. A.: Z. Elektrochem. **34,** 566 (1928).
(19) Lipsett, S. G., Johnson, F. M. G., and Maas, O.: J. Am. Chem. Soc. **49,** 925 (1927).
(20) Onsager, L.: Chem. Rev. **13,** 73 (1933).
(21) Redlich, O.: J. Phys. Chem. **44,** 619 (1940).
(22) Scatchard, G., and Prentiss, S. S.: J. Am. Chem. Soc. **55,** 4355 (1933).
(23) Scatchard, G., and Prentiss, S S.: J. Am. Chem. Soc. **56,** 1486, 2314, 2320 (1934).
(24) Scatchard, G., and Benedict, M. A.: J. Am. Chem. Soc. **58,** 837 (1936).
(25) Scatchard, G.: Chem. Rev. **19,** 309 (1936).
(26) Scatchard, G., Hamer, W. J., and Wood, S. E.: J. Am. Chem. Soc. **60,** 3061 (1938).
(27) Scatchard, G.: J. Chem. Phys. **7,** 657 (1939).
(28) Shedlovsky, T., and MacInnes, D. A.: J. Am. Chem. Soc. **59,** 503 (1937).
(29) Smith, R. P.: J. Am. Chem. Soc. **61,** 497 (1939).
(30) Smith, R. P., and Hirtle, D. S.: J. Am. Chem. Soc. **61,** 1123 (1939).
(31) Wyman, J., Jr., and Ingalls, E. N.: J. Am. Chem. Soc. **60,** 1182 (1938).

THE STABILIZATION OF VALENCES BY COÖRDINATION[1]

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Through the formation of complex ions of varying stability, the oxidation-reduction potentials of metal ions can be altered greatly. This often leads to a "stabilization of the valence" of the element in either higher or lower states of oxidation. Many factors which may have a bearing upon this phenomenon are considered, but it is pointed out that no theory of structure of complex compounds has yet been projected which adequately explains all of the very numerous examples of stabilization.

I. INTRODUCTION

The phrase "stabilization of valences by coördination" is used frequently in reference to the formation of ions in states of oxidation not ordinarily met in everyday laboratory practice. For example, silver salts may be oxidized to the divalent and trivalent states when coördinated with pyridine and analogous compounds (3, 13, 15, 19, 25), and iron may be carried to states of oxidation of 4+ and 6+ by anodic oxidation in alkaline solution (19). Cobalt ions exist ordinarily in aqueous solution in the divalent state, but in the well-known and stable ammines, cobalt is more stable in oxidation states of 3+ and perhaps 4+ (46). On the other hand, in the nitrosyl and carbonyl derivatives cobalt appears to exhibit oxidation numbers of -1 and 0 (5). Several studies have indicated that nickel (4, 6) and manganese (22) form complex cyanides in which the metals show quite unusual oxidation states. Coördination of the thermally unstable lead tetrachloride with two additional chloride ions, in hydrochloric acid solution, produces the much more stable hexachloroplumbate(IV) ion. The hydroxide ion coördinates strongly with many metallic ions, especially those of higher valence. Consequently, we usually achieve the higher valence states in alkaline solution (2). This tendency is doubtless furthered by the fact that the hydroxo complexes so formed lose water and are acidic, thus generating oxo complexes:

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$$[Cr(H_2O)_6]^{3+} \rightarrow [Cr(H_2O)_6]^{6+} \rightarrow [Cr(OH)_4]^{2+} \rightarrow [CrO_4]^{2-}$$

Carbon monoxide and nitric oxide always seem to stabilize the lower valences, and in the nitrosyls and carbonyls the metals appear in very low states of oxidation; in the volatile carbonyls and nitrosyls, in fact, the uncharged elements themselves combine with carbon monoxide and nitric oxide to form compounds in which their state of oxidation is zero. The non-volatile carbonyls are for the most part coördination compounds of carbon monoxide with salts in which the metal ion shows a low valence. Thus we have $PtCl_2 \cdot (CO)_2$, $RuI_2 \cdot (CO)_2$, $IrCl_2(CO)_2$, $PdCl_2(CO)$, and $OsCl_2(CO)_3$. Hieber and Marin (12) have even reported the preparation of $Co(NO)_2I$, in which cobalt shows a valence of 1. It is conceivable, of course, that the carbon monoxide or nitric oxide in these compounds assumes a negative charge from the metal, thus raising the formal charge of the latter beyond its apparent value. This idea was first suggested by Werner and Karrer (47), and has been developed by Reihlen (31, 32, 33, 34, 35). Manchot (21), however, has maintained that the carbon monoxide and nitric oxide retain their neutral character. Certainly the nitrosyls do not exhibit saltlike properties, and it is generally agreed that the metal shows an abnormally low valence.

II. OXIDATION-REDUCTION POTENTIALS

These are but striking manifestations of a completely general phenomenon. Uncoördinated metallic ions exist only in the gaseous phase; in solution, they at once coördinate with the solvent or with some other solute, and the coördinated ions thus generated have entirely different physical and chemical properties from the parent ions. As Emeléus and Anderson have pointed out (7b), "stabilization of valence" is simply a modification of the oxidation-reduction potential of the element or ion, induced by the specific influence of different coördinating groups. By coördination with water or ammonia or with chloride or bromide ion, the cupric ion forms complexes of greatly different color, and these complexes vary simultaneously in their resistance to reduction to the cuprous state or to the metal. This is a general phenomenon; coördination will nearly always (if not always) alter the tendency of an ion to undergo oxidation or reduction. When we say that coördination *stabilizes* an ion, we really mean (though we may not realize it) that the type of coördination in question produces a greater degree of stabilization than does *aquation*.[2] It should also be

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[2] *Hydration* means combination with water, but not necessarily to form hydrates; *aquation* refers to the formation of aquo ions; *aquotization* is more specific and refers to the expulsion of negative groups, from a complex ion, by the entrance of water molecules: e.g.,

$$[Co(NH_3)_5Cl]^{2+} + H_2O \rightarrow [Co(NH_3)_5(H_2O)]^{3+} + Cl^-$$

$$[CuBr_4]^{2-} + 4H_2O \rightarrow [Cu(H_2O)_4]^{2+} + 4Br^-$$

In this review, however, the more general term *aquation* will be used exclusively.

borne in mind that coördination may stabilize a valence state against reduction, but not against oxidation, or *vice versa.* In some cases, an ion is protected to some extent against a change in valence in either direction. Consideration of tables 1 and 2 will make these points clear.

It is evident from table 1 that ammoniation stabilizes the cobaltous state against reduction to the metallic condition more than does aquation but offers much less protection against oxidation to the cobaltic condition. Because we are accustomed to thinking in terms of aquated ions, the latter part of this

TABLE 1

Electrode potentials of some Co/Co^{2+} *and* Co^{2+}/Co^{3+} *couples (19)*

COUPLES	POTENTIALS
$[Co(CN)_6]^{4-} = [Co(CN)_6]^{3-} + e^-$	$E^0 = +0.83$
$Co + NH_3(aq) = [Co(NH_3)_6]^{2+} + 2e^-$	$E^0 = +0.422$
$Co + xH_2O = [Co(H_2O)_x]^{2+} + 2e$	$E^0 = +0.277$
$[Co(NH_3)_6]^{2+} = [Co(NH_3)_6]^{3+} + e$	$E^0 = -0.1$
$[Co(H_2O)_6]^{2+} = [Co(H_2O)_6]^{3+} + e$	$E^0 = -1.842$

TABLE 2

Electrode potentials of some Cu/Cu^{1+} *and* Cu^{1+}/Cu^{2+} *couples (19)*

COUPLES	POTENTIALS
$Cu + 2CN^{1-} = [Cu(CN)_2]^{1} + e$	$E^0 =$ *ca.* $+0.43$
$[Cu(H_2O)_x]^{1+} = [Cu(H_2O)_x]^{2+} + e^-$	$E^0 = -0.167$
$Cu + H_2O = Cu(H_2O)_x{}^{1+} + e^-$	$E^0 = -0.522$
$[Cu(CN)_2]^{1-} = [Cu(H_2O)_x]^{2+} + 2CN^- + e^-$	$E^0_B = -1.12$

TABLE 3

Electrode potentials of coördination Fe^{2+}/Fe^{3+} *couples (19)*

COUPLES	POTENTIALS
$[Fe(CN)_6]^{4-} = [Fe(CN)_6]^{3-} + e^-$	$E^0 = -0.36$
$[Fe(H_2O)_6]^{2+} = [Fe(H_2O)_6]^{3+} + e^-$	$E^0 = -0.771$
$[Fe(dipy)_3]^{2+} = [Fe(dipy)_3]^{3+} + e$	$E^0 =$ *ca.* -1.1
$[Fe(o\text{-}phen)_3]^{2+} = [Fe(o\text{-}phen)_3]^{3+} + e$	$E^0 = -1.14$
$[Fe(NO_2\text{-}o\text{-}phen)_3]^{2+} = [Fe(NO_2\text{-}o\text{-}phen)_3]^{3+} + e^-$	$E^0 = -1.25$

statement is usually worded in the converse: "Coördination with ammonia stabilizes the trivalence of cobalt against reduction (more than coördination with water does)." Coördination with the cyanide ion produces such a great change in the potential of the Co^{2+}/Co^{3+} couple that the hexacyano cobaltate (II) ion liberates hydrogen from water.

Table 2 illustrates the point that a donor group may protect an ion from both oxidation and reduction. In this case, coördination with the cyanide ion protects the cuprous ion against change in the oxidation state either way more than aquation does. Tables 3 and 4 show some similar relations for iron and cerium. It is evident that by varying the anion (since all of the acids are highly dissociated), it is possible to alter the composition and the stability of the complex which contains the cerium. Unpublished work done by Mr. R. C. Spooner in the laboratory of Professor Sherrill of the Massachusetts Institute of Technology (40) seems to indicate that the effect of the acid is possibly even more complex, owing to hydrolysis of the ceric ion, as shown by the half-cell reaction based entirely upon physicochemical data:

$$[\text{Ce, aq}]^{3+} + H_2O \rightarrow [\text{Ce}^{\text{IV}}(\text{OH})\text{ aq}]^{3+} + (H^+ + e^-)$$

Smith, Frank, and Getz, however, have chemical evidence to show that in the tetravalent condition cerium is present in a complex anion, such as $[Ce(NO_3)_6]^{2-}$ (42).

A valence state may seem to be stabilized, even though it is thermodynamically unstable, because it changes to another very slowly. We usually think that coördination with the cyanide ion protects the ferrous ion from oxidation, but the data of table 2 show that this is not the case; the tendency of ferrocyanide to undergo oxidation to ferricyanide is greater than that of the aquated ferrous ion to undergo oxidation to the aquated ferric ion. Many cases of "stabilization" should probably be explained as "decrease of oxidation (or reduction) rate." One cannot be certain until the electrode potential of the couple in question is known.

TABLE 4

Electrode potentials for the Ce^{3+}/Ce^{4+} couple in the presence of different acids 1 M in concentration (43)

Acid	$HClO_4$	HNO_3	H_2SO_4	HCl
E.M.F.	−1.70	−1.61	−1.44	−1.28

A. Metal-ion couples

In every consideration of the problem of stabilization it must be borne in mind that we seldom deal with ions which are *not* coördinated; simple ions do not exist in solution, but only in the gaseous or solid phases. Ions in solution are stabilized relative to the solid metal by solvation, thus in an aqueous solution by *hydration* or *aquation*. It is impossible to measure the absolute value of the energy difference between a solvated ion and the metal; however, the energy relative to the standard hydrogen couple is known for most of the metallic elements. The standard oxidation-reduction potential, as usually defined, is a measure of this relative energy difference for a couple comprised of the metal and the aquated ion at unit activity. For smaller *activities* of the aquated ion, the oxidation-reduction potential has a larger positive (or smaller negative) value, and when the *activity* is increased, the potential becomes less positive (or more negative), in accordance with the equation:

$$E = E^0 - \frac{0.05914}{n} \log_{10} Q$$

where Q has the same general form as the equilibrium constant but refers to the actual activities of the reaction substances and their products (19). If a coördinating group—ion or molecule—is added to a solution containing the aquated ions, with the result that the water of aquation is largely replaced, the *activity* of the hydrated ion is reduced, and the new complex ion is more stable relative to the metal than the aquated ion. The standard oxidation–reduction potential for the new couple has a larger positive (or less negative) value: that is, the new complex ion formed is a poorer oxidizing agent than the aquated ion. This is illustrated in the behavior of copper with hydrochloric, hydrobromic, and hydriodic acids, as shown in table 5. The oxidation–reduction potential of the metallic copper-cuprous couple is raised sufficiently by the more strongly coördinated bromide and iodide ions to permit the displacement of hydrogen from the concentrated acids, but not with hydrochloric acid under ordinary conditions of temperature and concentration.

Similar changes in electrode potentials are observed with all the other elements and must be taken into account in predicting the course of chemical reactions (19).

TABLE 5

Electrode potential for the Cu/Cu^+ *couple in the presence of halide ions (19)*

COUPLES	POTENTIALS
$aq + Cu = [Cu, aq]^+ + e^-$	$E^0 = -0.522$
$2Cl^- + Cu = [CuCl_2]^- + e^-$	$E^0 = -0.19$
$2Br^- + Cu = [CuBr_2]^- + e^-$	$E^0 = -0.05$
$2I^- + Cu = [CuI_2]^- + e^-$	$E^0 = 0.00$

B. Polarographic determination of oxidation–reduction potentials

The polarographic method of analysis gives a measure of oxidation–reduction potentials, and has brought to light many examples of valence stabilization through complex-ion formation. The half-wave potential of the ferric ion found by this method is -1.32 volts, but addition of excess oxalate ion converts this to a value so strongly negative that it cannot be measured. Addition of fluoride ion produces a similar effect. The complex ions formed are apparently $[Fe(C_2O_4)_3]^{3-}$ and $[FeF_6]^{3-}$ (44). The aquated cupric ion gives only one polarographic wave, which corresponds to reduction to the metal, but cupric solutions containing ammonia or chloride ion show two distinct waves, the first corresponding to reduction to a stabilized cuprous state (44). Pines, using the polarographic technic in the study of the deposition of zinc from cyanide solutions, has found that if the ratio of potassium cyanide to zinc cyanide is less than 4:1, zinc will deposit. Larger amounts of potassium cyanide cause the formation of $K_4Zn(CN)_6$, from which potassium, rather than zinc, is electrodeposited (28). Cadmium, on the other hand, is deposited from cyanide solutions, no matter how great the excess of cyanide ion (29).

Considerations of this sort have found practical application in the polarographic estimation of related ions. The half-wave potentials of the aquated zinc and nickel ions are so close together that the simultaneous determination of these ions, as such, is impossible. The addition of a large excess of ammonium oxalate, however, causes the formation of complex oxalato ions which differ greatly in their resistance to reduction, and can be readily estimated (30). The data of Lingane and Kerlinger (20) on the similar case of nickel and cobalt are interesting in this connection (see table 6). The solutions used were 0.001 *M* in nickel(II) chloride and 0.002 *M* in cobalt(II) chloride and contained 0.01 per cent of gelatin. Half-wave potentials are given with respect to the calomel electrode at 25°C. It is interesting to observe that the addition of pyridine or thiocyanate ion, both of which are coördinated strongly, increases the ease with which the nickel and cobalt ions are reduced (but not equally). It is well known that the presence of these substances also increases the ease with which the cobalt(II) ion is oxidized.

Heyrovsky and Ilkovič (11) assume that reduction of a complex ion takes place in two steps: (*1*) formation of the simple ion and (*2*) its subsequent reduction. This is doubtless an oversimplification of the process, for the relationships between the oxidized and reduced forms of the metal ion, the coördinating group, and the solvent (which in most cases can also form complexes with both forms of the metallic ion) must be extremely complex. If their assumption is correct, stabilization of the ion against reduction takes place only because the concentration of reducible ion is greatly decreased. Kolthoff and Lingane (18), in a review of this field, have suggested that "the reduction of very stable complex metal ions may take place by the direct capture of electrons from the electrode".

TABLE 6

Half-wave potential differences due to coördination (20)

ELECTROLYTE ADDED	CONCENTRATION	HALF-WAVE POTENTIALS		
		Ni^{2+}	Co^{2+}	Difference
	M			
KCl	1	−1.1	−1.2	0.1
NH_4Cl NH_4OH	1 1	−1.12	−1.30	0.18
KCl C_5H_5N	1 0.5	−0.78	−1.07	0.29
KCNS	1	−0.70	−1.03	0.33

Ion stabilization is thus directly connected with the equilibrium constant for the aquation of the complex ion. The smaller the value of this constant, the more stable the ion is relative to the metal.

C. Ionic oxidation–reduction couples

When we come to a comparison of the stability in solution of two different valence states of a metal, we may still use oxidation–reduction potentials as criteria. The oxidation–reduction potential of a couple comprised of the ions in the two valence states, each at unit activity and presumably aquated, in contact with an inert electrode, is used as a standard. Addition of another coördinating group will in general produce *unequal* changes in the concentration or activities of the aquated ions in the two oxidation states. If the displacement of the water of aquation proceeds nearer to completion for the higher state of oxidation— thus, if in the higher state of oxidation the newly coördinated ion is more stable than in the lower state— then the oxidation–reduction potential of the new couple will have a higher positive (or less negative) value. As has been shown in table 1, the oxidation-reduction potential of the couple $[Co(H_2O)_6]^{2+}/[Co(H_2O)_6]^{3+}$ is -1.842 volts and, as a result, the aquated cobalt(III) ion is a powerful oxidizing agent; on the other hand, the oxidation-reduction potential of the complex cyanide couple $[Co(CN)_6]^{4-}/[Co(CN)_6]^{3-}$ is $+0.83$ volt. The hexacyanocobaltate(II) complex is thus a powerful reducing agent and even reduces water with the evolution of hydrogen. Contrariwise, the couple $[Fe(H_2O)_6]^{2+}/[Fe(H_2O)_6]^{3+}$ has an oxidation-reduction potential of -0.771 volt, compared to that of -1.14 volts for the couple comprised of the ferrous and ferric ions coördinated with *o*-phenanthroline, $[Fe(C_{12}H_8N_2)_3]^{2+}/[Fe(C_{12}H_8N_2)_3]^{3+}$. Evidently the *o*-phenanthroline forms a more stable complex with divalent than with trivalent iron. The trivalent state, however, is stabilized by coördination with α-pyridylhydrazine (9) and α-(α′-pyridyl)pyrrole (8). It is of interest to note that all good oxidation–reduction indicators for the titration of ferrous ion are examples of coördination in which stabilization of low valences is achieved.

Hence the equilibrium constants for the aquation of the corresponding complexes of an element in two states of oxidation give us a measure of the resistance to change in valence. If the equilibrium constant for the higher valence state is smaller than that for the lower valence state, stabilization of the higher valence state results, since this involves a greater decrease in the concentration of the aquated ion in the higher valence. This line of reasoning suggests that certain predictions as to the ease of oxidation or reduction can be made. Thus, we should expect the chloropentammine cobalt(III) ion to be reduced more readily than the nitropentammine or the hexammine, for it is readily aquated while the others are not.

An interesting application of these concepts has been made by Reinders and his coworkers (36, 37, 38). They have shown that the oxidation-reduction potential of an equimolar mixture of ferrous and ferric sulfates can be varied from $+0.64$ volt to zero by the addition of salts of hydroxy- or poly-carboxylic organic acids. The salts which they studied were sodium citrate, malonate, oxalate, tartrate, lactate, and succinate, of which the first three were the most satisfactory. The anions of these salts coördinate with both the ferrous and ferric ions and give solutions which show a considerable buffer action toward oxidation and reduction. These systems were used in studying the process of photographic development.

III. TYPES OF BONDS FOUND IN COÖRDINATION COMPLEXES

In a complex, each atom loses its individual properties, including its characteristic state of oxidation, and becomes simply a part of the whole. The resultant electrical charge of a complex ion is determined by the coördinating groups as well as by the central metal ion. It is quite possible that any metal could be carried through a wide range of stable oxidation states if it were coördinated with the proper groups. Conversely, it is evident that with many elements the realization of different states of oxidation is possible only through coördination with certain atoms or groups of atoms. A most important question arises: "Why do different coördinating groups, apparently sharing the same number of electrons with a metallic ion, give rise to complexes of such varying electron affinities?" No complete answer to this question has yet been offered—indeed, none seems possible in our present state of knowledge. It is the purpose of this review to discuss certain aspects of the problem with the hope of stimulating further study of it.

Sidgwick (41) has suggested that stable valence groups are formed when the *effective atomic number* of the metallic ion is equal to that of an inert gas. On this basis, the stability of the hexammine cobaltic salts is attributed to the fact that the twenty-four electrons of the metal ion, with the twelve furnished by the coördinated nitrogen atoms, give the stable inert-gas configuration of krypton. The stability of $Co(CO)_3NO$, in which the cobalt atom seems to have no charge, is explained in the same way $[27 + (3 \times 2) + 3 = 36]$. This theory may appear to explain the formation of many coördination compounds, but there are numerous exceptions to it. We cannot hope to use it to explain the stabilization of oxidation states by coördination when different groups, which apparently furnish the same number of electrons, do not stabilize the same valence state. Thus, coördination with *o*-phenanthroline stabilizes the divalency of iron (in this case the *E.A.N.* becomes that of krypton) but coördination with water, cyanide, or hydroxyl groups stabilizes the trivalency, even though the resultant complex has one electron fewer than the inert gas.

Fajans has attempted to explain the instability of cupric iodide on the basis of *ion-deformation* (10). The deepening in color of the silver and cupric halides with increasing atomic weight of the halogen indicates an increasing deformation of the electron shells of the halogen. The iodide ion is so greatly deformed by its proximity to the cupric ion that it completely loses one of its electrons, with consequent reduction of the cupric ion. This explanation implies that the cupric condition will be more stable if the metallic ion is kept from close proximity to the iodide ion, by surrounding it, say, with coördinated neutral groups. The diethylenediamine copper(II) ion, $[Cuen_2]^{2+}$, in contrast to the aquated cupric ion, indeed forms a stable iodide, and is not reduced by hyposulfites (23). On the other hand, the cuprous condition of copper is stabilized by coördination with acetonitrile (26) and derivatives of thiourea and thiocarbamide (17, 24, 39).

One of the common pitfalls in the consideration of the problem of stabilization of valences should perhaps be mentioned here. It must be borne in mind that the *apparent* oxidation state of an element is not always a true measure of its condition. Thus, the supposed divalency of gallium, indium, and thallium is very doubtful, the dichlorides probably being auto-complexes of the type $Ga^{I}[Ga^{III}Cl_4]$, $In^{I}[In^{III}Cl_4]$, and $Tl^{I}[Tl^{III}Cl_4]$ (7a). The diamagnetic nature of these dihalides (16) is in agreement with such a formulation. We can safely draw conclusions only when it is clearly demonstrated that the complex in question has a known structure and contains only one ion or atom of variable valence,—conditions frequently not fulfilled.

Coördinating groups, presumably, must have an atom present with at least one pair of unshared electrons, but the type of bond formed is not at once apparent. The attraction of the field around a positive ion for the pair of electrons might be considered to be largely responsible for the formation of a coördinate bond. The intensity of this field surrounding the ion depends on its charge and is obviously always greater for the more highly charged valence state. If the bond between the coördinating group and the ion were of ionic character, that is, only so-called Coulombic forces were operative, then the stronger bond would always be formed by the more highly charged ion. This would lead to a uniformly smaller value for the aquation constant in the higher valence state and thus stabilization of the higher valence state. The numerous examples of stabilization of low valences contradict the assumption of a purely ionic bond and suggest that, in general, the coördinate link is covalent in character. Evidence for the existence of covalent bonds in many coördination complexes has been obtained from the measurement of the magnetic moments of ions in solution (27a). On the basis of the available information practically the only coördinating groups which form bonds of ionic character are water, fluoride ion, and occasionally the oxalate ion. Very few quantitative data are available on fluoride and oxalate complexes; these are, however, sufficient to allow the conclusion to be drawn that in the fluoroferrate and oxalatoferrate ions the trivalent state is stabilized. The resolution of many coördinated ions into optical isomers suggests, in addition, that covalent bonds occur frequently, since if ionic bonds were present it is less likely that definite orientation in space relative to the central ion would exist.

An excellent discussion of the type of bonds involved in the formation of complex ions is given in a recent treatise by Pauling (27b), in which he elaborates the idea that "atoms in the transitional groups are not restricted to the formation of single covalent bonds but can form multiple covalent bonds with electron-accepting groups by making use of the electrons and orbitals of the shell within the valence shell". Because of this it is difficult to postulate the formal charge or state of oxidation of the central atom. It would not seem possible at present, however, to explain on the basis of multiple bonds why zinc forms complexes with cyanide ions while aluminum and gallium do not. The specific and individual behavior of many of the elements still lacks clarification.[3]

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[3] *Note added in proof:* J. E. B. Randles (J. Chem. Soc. **1941,** 802) has investigated the factors governing the stability of cuprous and cupric complexes and claims that, "The chief controlling factor appears to be the degree of covalency or electrovalency of the bonding between the copper ions and neighbouring ions or molecules, which is mainly dependent on the polarizability of the latter." This concept does not appear to be of general application, however, since hydration does not invariably form the higher state of oxidation, nor does combination with ammonia or pyridine invariably lead to stabilization of the lower state, as has been emphasized in the body of this review. Randles' conclusions, while in agreement with the experimental data he presents, cannot be extended to other systems.

IV. CONCLUSIONS

When the element in two states of oxidation forms covalent bonds with the coördinating groups, our present knowledge of the factors which govern this type of bond formation does not permit the prediction of bond strengths; hence no theoretical estimate of the relative magnitudes of the aquation constants for different valence states can be made. The experimental evidence from aquation constants and from oxidation–reduction potentials leads to the following conclusions:

1. *If, for an element in a particular state of oxidation, its complexes with coördinating groups are listed in the order of increasing stability, it is probable that the same order will not hold for other valence states of the element.* There seem to be no definite examples of actual changes in order, but there are many examples of large changes in stability, so it is logical to assume that the order may be altered. There is need for more data on this point.

It may be difficult to obtain such data, however, for any great change in stability may make the ion so unstable that it will not exist under ordinary conditions. In other words, the difference between "stability" and "instability" is not great, and any large change in conditions may forbid an oxidation state completely, thus making it impossible to obtain some of the complexes which it would be desirable to study. An example of this is afforded by the triethylenediamine chromium(II) ion, which apparently cannot exist in water solution but changes rapidly to the corresponding chromium(III) ion (1) with the evolution of hydrogen. Hydrazine, however, gives fairly stable complex chromium(II) ions (45).

2. *Different ions in the same state of oxidation do not exhibit the same order of stability with coördinating groups. Thus, it is impossible to arrange coördinating groups in a definite series, in the order of their ability to form stable bonds, which will apply to all metallic ions.* However, certain rough generalizations may be made from available data. Familiar coördinating groups, such as hydroxyl ion, cyanide ion, nitrite ion, and ammonia, ordinarily stabilize the higher valence state. There seem to be some exceptions to this, such as the compounds $K_4Ni^0(CN)_4$ and $K_2Ni^I(CN)_3$, recently discussed by Burgess (6), and $K_6Mn^I(CN)_6$, reported by Manchot (22). These compounds, however, have not been completely studied, and when their structure has been determined it is possible that the apparent discrepancies will disappear. If they are real exceptions, it must be admitted that at present there is no explanation for them.

A survey of the various types of stabilization groups seems to substantiate a rule which has been suggested by Professor John P. Howe of Brown University (14): *Coördinating groups of higher negativity stabilize the higher valences, and large groups containing resonating structures stabilize the lower valence states;* thus, divalent oxide ions stabilize chromium, iron, and nickel with an oxidation number of 6, as in $[CrO_4]^{2-}$, $[FeO_4]^{2-}$, and $[NiO_4]^{2-}$, and, as previously noted, other negative ions in general stabilize higher valences. Neutral molecules like *o*-phenanthroline, with conjugated double bond structures, stabilize the lower valences. This might be summarized by stating "that the greater the electron affinity of the group itself for additional electrons, the more the lower valence state is stabilized".

V. SUMMARY

Valence stabilization by coördination is a real phenomenon, but the term signifies a relatively large change in the oxidation-reduction potential of a couple, rather than a sudden and unexpected jump in valence. The phenomenon is a very general one, but at present no adequate theory of structure of complex compounds is able to explain much of the data. Experimenters who measure electrode potentials are cautioned to ascertain and to report *all* of the components of their solution, as frequently the obscure variations in potentials observed are due to the formation of complexes which have been overlooked. Many of the data on electrode potentials recorded in the literature are worthless because of this oversight.

REFERENCES

(1) Balthis, J. H., Jr., and Bailar, J. C., Jr.: J. Am. Chem. Soc. **58,** 1474 (1936).
(2) Bancroft, W. D.: J. Chem. Education **11,** 267 (1934).
(3) Barbieri, G. A.: Atti accad. Lincei [5] **15,** i, 500 (1906); [6] **13,** 882 (1931); **16,** 44 (1932); **17,** 1078 (1933); Ber. **60,** 2424 (1927).
(4) Belucci, I., and Corelli, R.: Atti accad. Lincei [5] **15,** i, 500 (1906); **22,** i, 603 (1913); **22.** ii, 485 (1913); Gazz. chim. ital. **43,** ii, 569 (1913); **49,** ii, 70 (1919); Z. anorg. Chem. **86,** 88 (1914).
(5) Blanchard, A. A.: Chem. Rev. **21,** 3 (1937).
(6) Burgess, W. M.: Abstracts of Papers, Division of Physical and Inorganic Chemistry, American Chemical Society, Boston, September, 1939, pages 36-37.
(7) Emeléus, H. J., and Anderson, J. S.: *Modern Aspects of Inorganic Chemistry*: (a) p. 145; (b) p. 151. D. Van Nostrand Company, New York (1938).
(8) Emmert, B., and Brandt, F.: Ber. **60,** 2211 (1927).
(9) Emmert, B., and Schneider, O.: Ber. **66,** 1875 (1933)
(10) Fajans, K.: Naturwissenschaften **11,** 165 (1923).
(11) Heyrovsky, U., and Ilkovič, D.: Collection Czechoslov. Chem. Commun. **7,** 198 (1935).
(12) Hieber, W., and Marin, R.: Z. anorg. allgem. Chem. **240,** 241 (1939).
(13) Hieber, W., and Mühlbauer, F.: Ber. **61,** 2149 (1928).
(14) Howe, J. P. (Brown University, Providence, Rhode Island): Private communication.
(15) Klemm, W.: Z. anorg. allgem. Chem. **201,** 32 (1931).
(16) Klemm, W., and Tilk, W.: Z. anorg. allgem. Chem. **207,** 175 (1932).
(17) Kohlschütter, V.: Ber. **36,** 1151 (1903).
Kohlschütter, V., and Brittlebank, C.: Ann. **349,** 232 (1906).
(18) Kolthoff, T. M., and Lingane, J. J.: Chem. Rev. **24,** 1 (1939).
(19) Latimer, W. M.: *Oxidation Potentials.* Prentice-Hall, Inc., New York (1938).
(20) Lingane, J. J., and Kerlinger, H.: Ind. Eng. Chem., Anal. Ed. **13,** 77 (1941).
(21) Manchot, W.: Ann. **459,** 47 (1927).
(22) Manchot, W., and Gall, H.: Ber. **60,** 191 (1927); **61,** 1135 (1928).
(23) Morgan, G. T., and Burstall, F. H.: J. Chem. Soc. **1926,** 2018, 2027; **1927,** 1259.
(24) Morgan, G. T., and Burstall, F. H.: J. Chem. Soc. **1928,** 143.
(25) Morgan, G. T., and Burstall, F. H.: J. Chem. Soc. **1930,** 2594.
(26) Morgan, H. H.: J. Chem. Soc. **123,** 2901 (1923).
(27) Pauling, L. C.: *The Nature of the Chemical Bond*: (a) pp. 104-11; (b) pp. 231-8. Cornell University Press, Ithaca, New York (1939).

(28) Pines, I.: Collection Czechoslov. Chem. Commun. **1**, 429 (1929).

(29) Pines, I.: Collection Czechoslov. Chem. Commun. **1**, 387 (1929).

(30) Prajzler, J.: Collection Czechoslov. Chem. Commun. **3**, 406 (1931).

(31) Reihlen, H.: Z. anorg. allgem. Chem. **230**, 223 (1937).

(32) Reihlen, H., and Friedolsheim, A.: Ann. **457**, 71 (1927).

(33) Reihlen, H., Friedolsheim, A., and Oswald, W.: Ann. **465**, 72 (1928).

(34) Reihlen, H., Gruhl, A., and Hessling, G. v.: Ann. **472**, 268 (1929).

(35) Reihlen, H., Gruhl, A., Hessling, G. v., and Pfrengle, O.: Ann. **482**, 161 (1930).

(36) Reinders, W.: J. Phys. Chem. **38**, 783 (1934).

(37) Reinders, W., and Beukers, M. C. F.: 9^{me} Intern. Congress Photogr., Paris (1935), p. 345.

(38) Reinders, W., and de Minjer, C. H.: Rec. trav. chim. **57**, 594 (1938).

(39) Rosenheim, A., and Lowenstamm, W.: Z. anorg. Chem. **34**, 62 (1903).

(40) Sherrill, M. S.: Private communication.

(41) Sidgwick, N. V.: *The Electronic Theory of Valency*, pp. 163 ff. Oxford University Press, London (1929).

(42) Smith, G. F., Frank, G., and Kott, A. E.: Ind. Eng. Chem., Anal. Ed. **12**, 268 (1940).

(43) Smith, G. F., and Getz, C. A.: Ind. Eng. Chem., Anal. Ed. **10**, 191 (1938).

(44) Stackelberg, M. von, and Freyhold, H. von: Z. Elektrochem. **46**, 120 (1940).

(45) Traube, W., and Passarge, W.: Ber. **46**, 1505 (1913).

(46) Werner, A.: Ann. **375**, 15 (1910).

(47) Werner, A., and Karrer, P.: Helv. Chim. Acta **1**, 54 (1918).

RECENT WORK IN MOLECULAR BEAMS

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The production and detection of molecular beams is described briefly. Then follows a discussion of a number of recent problems which have been solved wholly or partially by the application of molecular-beam technique. Special emphasis is placed on the determination of the magnetic properties of atomic nuclei, and a rather complete table of nuclear moments is given.

I. INTRODUCTION

It is hoped that the following résumé of some of the more important recent work in molecular beams will be of interest, not only to those who have specialized in this field, but also to others. It is our conviction that to some of these latter the molecular-beam method will appear especially appropriate for the solution of new problems. The method has been very fruitful in the past, and there is every reason to expect that as more and more people become proficient in its use, a greater and greater variety of problems will be successfully solved.

The basis of the molecular-beam method lies in the kinetic theory of gases. This is a very beautiful and satisfying theory. Developing gradually, it had acquired such distinction by the first decade of this century that very few people doubted its main features. In brief, these are that all gases are composed of particles moving constantly in straight lines and continually colliding with each other and with the walls of the confining vessel. The speeds of these particles will in general change with each collision; therefore there will be a wide range of velocities in every gas sample. However, at a constant temperature there will be an average velocity which does not change with the time and which can be easily calculated. Furthermore, it is a very simple matter to obtain an expression for the average distance each molecule travels between collisions; this mean free path turns out to be inversely proportional to the pressure.

From these simple assumptions was developed an elegant theory, which, although not directly tested, gave predictions sufficiently close to experimental results to satisfy almost everyone. Deviations from the theory were explained qualitatively as due to the neglect of the finite sizes of the particles and the forces of interaction between them.

Nevertheless, it is always a very satisfying procedure to make a direct test of fundamentals. As long ago as 1895 (4), manufacturers of incandescent light bulbs found evidences of the rectilinear propagation of gaseous particles. Sharp shadows in the evaporated coatings on the insides of old bulbs could be explained only by assuming such a straight-line motion. However, they performed no experiments for the purpose of testing this point alone. This was first done in 1911 by Dunoyer (37), who heated some sodium in the bottom of an evacuated tube with several collinear constrictions in it. He found that the condensate at the other end was very sharply defined and had exactly the shape expected under the assumption of rectilinear motion, just as in the corresponding optical case. To test the matter further, he placed peculiarly shaped obstacles in the beam path and found shadows, sharp and clear, of the same shape and size. He suggested that this method might be used to test other fundamental ideas of the kinetic theory, but did not seriously attack the problem himself.

Stern (235), in 1920, adapted Dunoyer's "one-dimensional gas" to the simple and direct measurement of the average velocity of the atoms of silver in the vapor state. This was the first of a long and brilliant series of experiments with what he preferred to call atomic or molecular rays. Although this first measurement was rather crude, yet it was sufficiently precise to give the kinetic theory value for the average velocity, to within experimental error. Soon thereafter, Born (14, 19), using a well-defined beam of metallic atoms, obtained estimates of their mean free path in a gas at low pressure. His results showed that the product of the pressure and the mean free path was a constant, as expected.

Many more experiments have been performed with molecular rays since these three which established in such a beautiful and direct manner the fundamental concepts of the kinetic theory. The apparatus is still built like Dunoyer's, with improvements, of course. The basic idea is still the same: to produce a beam of particles of small cross-sectional area, all the members of which are moving in essentially the same direction in a space where the pressure is sufficiently low so that these individuals do not collide with each other or with other atoms. If one does something to the beam (as, for example, passing it through a force field), the results will simply be the statistical sum of performing that same act upon each particle in the beam, independently of all the rest. Although a single atomic process is not in general observable, if the beam is sufficiently intense the accumulative effect will be measurable. Thus the molecular-ray method provides the possibility of observing atomic behavior directly, and has consequently been applied to the solution of many problems.

The distribution of velocities in a beam has been analyzed and found to be in agreement with the theoretical prediction. Beams have been scattered in various gases, and the mean free paths and collision cross sections determined. Various interactions between atoms and surfaces have been investigated.

It was pointed out by Stern (238) that, if the ideas of de Broglie on the wave nature of matter were correct, then under the proper conditions a beam of particles should be diffracted similarly to a beam of light. The wave length λ associated with a molecule of mass m and velocity v is determined from the relation $\lambda = (h)/(mv)$, where h is Planck's constant. The molecular-beam method has been used many times to test this theory and offers the only direct confirmation of it in the case of uncharged particles.

In 1921, Stern (236) suggested that the molecular-beam method could be used as a crucial test to decide between the classical theory and the quantum theory predictions for the magnetic properties of atoms and molecules. If a beam of atoms, each with a magnetic moment, is passed through an inhomogeneous magnetic field, the direction of the inhomogeneity being essentially parallel to the field, the angular momentum vector of each atom will precess about this direction, and the atom will be deflected by the magnetic force. The magnitude of the deflection will be proportional to the projection of the moment upon the field direction. According to the classical theory, the magnetic moment may be oriented in any direction whatsoever, so that its projection might be anything between zero and the absolute value of the moment itself. Then there would be deflections of all magnitudes, and the beam would merely be broadened. According to the quantum theory, on the other hand, there are only a few possible orientations of the moment with respect to the field, and consequently there will be a splitting of the beam into discrete components. The case of silver is a good example. If it behaves classically, a beam of silver atoms will be broadened, but with the position of maximum intensity still located at the position of the undeflected beam. If, on the contrary, the quantum theory applies, since according to this theory there are only two possible orientations of the moment axis, the beam will be split into two parts with a minimum of intensity at the undeflected position. An experiment of this type was performed by Gerlach and Stern (80). They passed a beam of silver atoms through a strong field produced by a powerful electromagnet with pole pieces shaped like a slot and a wedge. The results confirmed completely the predictions of the quantum theory. This brilliant and spectacular experiment was the forerunner of a large number of precise measurements of the magnetic properties of atoms and molecules.

There have been many other applications of molecular rays, some of which will be discussed more in detail in the following. This brief description has perhaps served to indicate the power and scope of the method. Aside from its simplicity and directness, it has the obvious advantage that any external applied forces can be made to act on molecules the positions and directions of motion of which are known. Furthermore, there are no mutual interactions in the beam to produce spurious effects. In addition, the molecular-beam method is particularly useful in the measurement of exceedingly small nuclear quantities (see footnote, page 255).

II. THE PRODUCTION AND DETECTION OF MOLECULAR BEAMS

A gas at low pressure is allowed to escape through an orifice or slit into a highly evacuated space. The emerging particles will move out radially from this slit, travelling in straight lines until they strike an obstruction. If a diaphragm containing another small opening is placed in front of the first slit, those particles which pass through will all be moving in essentially the same direction; that is, a beam of unidirectional particles has been selected out of the randomly moving ones. The apparatus thus consists of a source chamber or oven, a series of collinear slits to define the beam, and a detector. To prevent scattering of the beam particles, the apparatus must be highly evacuated and the oven slit must not be too large.

Beams are made of permanent gases or the vapors of condensed substances. The condensed matter is enclosed in a small oven or furnace in which there is an oven slit. The oven is heated until the vapor pressure of the contents reaches a value great enough to produce a beam of the desired intensity. In order to prevent scattering within the apparatus, large cooled surfaces are supplied to freeze out any molecules not in the main beam itself. The technique is a little more complicated when the permanent gases are used. In this case there must be a constant supply of the gas at a predetermined low pressure. Because these gases can not be condensed when they are scattered out of the beam but will be reflected diffusely at the walls of the apparatus and thus increase the residual pressure, it is necessary to use very high speed pumps.

In order to produce a true molecular beam one must have a sufficient number of slits to define it. This means that at least two must be used. Sometimes it is found more advantageous to use three; this is especially helpful in the case where the beam is made of a non-condensable gas. These slits are made of metal or glass and are aligned approximately by optical or mechanical means. In experiments where questions of intensity arise or where precise quantitative measurements are desired, the final adjustment of the slits is often made by observation of the effect of moving them upon the intensity and shape of the beam itself.

The first beams were made with metals of relatively low melting point. These were easily purified and vaporized, and their detection offered no difficulties. All that was necessary was to interpose a cooled plate in the path of the beam. Many investigations[1] have been made concerning the effect of beam pressure and temperature upon the nature of the trace produced. It was even found possible by either physical or chemical methods to develop a trace which was too faint to be seen. Since many deposits are destroyed upon contact with the air, devices have been used to photograph them in the vacuum.

It was soon found that certain beams could be detected by their chemical activity. For example, atomic hydrogen beams will reduce the yellow oxide of molybdenum to the blue one, or will blacken the Schumann plate (128). These are the so-called accumulative methods and are very sensitive; if one waits long enough the result is almost certain to be visible. However, there are serious disadvantages. There are many substances which can be neither condensed nor detected chemically. Furthermore, because the molecules in a thin film have a tendency to move sideways along the supporting surface, the reproducibility is poor, and there is no possibility of obtaining quantitative results.

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[1] For a complete discussion see R. G. J. Fraser: *Molecular Rays*, University Press, Cambridge (1931).

Various investigators have weighed the deposits or analyzed them with a microphotometer, but without much success.

Since several other methods have been developed which will give quantitative results, these early ones have been largely superseded. However, very recently Simons and Glasser (231) have perfected a sensitive chemical detector in the form of a tellurium oxide; it will react with atomic hydrogen, oxygen, chlorine, and bromine, and perhaps with some of the organic free radicals.

The first reliable quantitative detector was the ionization gauge, used by Johnson (108) in 1926. He found that it did not behave well, but several other investigators have since used it for relatively heavy molecules with complete success. Stern and Knauer (132) constructed a very sensitive Pirani gauge or hot-wire manometer. It is very useful for the light permanent gases, but its sensitivity decreases with increasing molecular weight. These two gauges measure simply the pressure built up in the manometer by the beam. Radiometer vanes (30, 32, 161), bolometers (254), and thermopiles (unpublished work by various investigators) have been used, but have never proven entirely satisfactory.

In 1929, Taylor (243) developed a quantitative detector for atoms with low ionization potentials, based upon Langmuir's investigations of surface ionization. It is useful for the alkali metals, either as atoms or molecules, and for a few other substances such as indium and gallium; it will also detect beams of alkali salts (209) and other compounds containing the alkali-metal atoms. As customarily used, this gauge consists of a hot tungsten filament, sometimes with an oxide coating, and a negative plate. A definite fraction (usually practically 100 per cent) of all the atoms which strike the wire are ionized and repelled to the negative collector. The positive-ion current is thus a measure of the intensity of the beam. Rabi and Cohen (196) have developed a modification of this regular procedure for cases in which the intensity is extremely low. They allow the atoms to strike and to accumulate upon a cold wire which is flashed at regular intervals. The total charge received by the collector is proportional to the total number of atoms arriving at the detector during the whole time. Tests show that this accumulative method is very reliable, even for periods of collection of as much as 10 min. Kodera (135) has recently used a modification of this gauge to measure the total intensity of very broad beams.

Langmuir and Villars (147) have suggested that it might be possible to use a tungsten wire to detect beams of oxygen. Electron emission from such a wire, activated by small amounts of cesium vapor, is very sensitive to changes in the oxide coating. As far as the authors know, no one has attempted to make use of this idea.

All of the above quantitative instruments, although very good for a few molecular species, have the disadvantage of being relatively insensitive for the majority of beam materials. In 1933 Estermann and Stern (59) constructed a universal gauge of extremely great sensitivity. It is based on the investigations of Kingdon and Langmuir on the elimination of space charge by positive ions, and consists simply of a hot filament surrounded by a positive cylinder. If the filament temperature and anode potential are adjusted properly, and if there is a good vacuum between the electrodes, the current will be limited by the space charge. If now a gas is admitted to the gauge, some of the gas molecules will be ionized by the electrons. These ions will spiral round and round the filament, thus effectively decreasing the space charge; this will produce a large change in the plate current, the amount of change depending of course upon the number of ions present. Kingdon used a device of this type (the Kingdon cage) to measure small pressures. It is extremely sensitive because the electron current can be amplified by appropriate means.

Using this device, Stern and Estermann made some rough experiments with a mercury beam, and were able to measure the beam intensity when the mercury oven was kept at $-30°C$. They used as a filament a thorium-free tungsten wire. The positive plate was a cylinder of nickel sheet capped at both ends; the beam passed in through a hole in one of the caps. A second gauge exactly like the first was used to balance out fluctuations in residual pressure, pumping speed, and the like. A sensitive galvanometer measured the difference between the two plate currents. It was found that there was an optimum value of the anode potential for which the sensitivity was a maximum. This was, of course, greater than the ionization potential of mercury. It was also discovered that for extremely low pressures the galvanometer throw was not exactly proportional to the beam pressure as determined from the temperature of the oven. This means either that the gauge must be placed in a room with a small residual pressure so that it will give linear readings, or else that the gauge must be calibrated carefully over its whole range if it is to be used in a good vacuum.

This is a universal method, since all substances which can be made into beams can be ionized by swift electrons. It has been pointed out by Fraser (68) that it would be useful for investigating the organic free radicals. It is extremely sensitive; however, the sensitivity is greater for the heavier molecules, since they have a smaller velocity and will therefore remain in the space charge region for a longer time. In spite of these very obvious advantages, this method has been used very infrequently.

III. VELOCITY DISTRIBUTION IN MOLECULAR BEAMS

One of the consequences of the kinetic theory is that the molecules in a molecular beam do not all have the same velocity. This complicates considerably the interpretation of a molecular-beam experiment. For example, if a beam is passed through a force field, there will be a different deflection for every velocity. Thus, it is usually desirable and frequently necessary to know the distribution of velocities in a beam.

Three methods have been used in velocity measurements: mechanical selection, deflection in a force field, and selective reflection. Costa, Smyth, and Compton (32), Eldridge (39), and Lammert (146) have used the toothed-wheel method of measuring the velocities of beam particles. Similar to the old Fizeau method of measuring the velocity of light, it consists in passing a beam through two or more coaxial rotating notched disks. From a knowledge of the number of teeth, the speed of rotation, and the distance between the wheels, one may easily calculate the velocity of the particles which are able to pass through. Zartman (263) and later Ko (134) used a refinement of the Stern method (235) of measuring average velocities. Their experiments were performed with a claimed precision sufficient to indicate more slow particles in a beam of bismuth than were predicted by the theory. This result they explained by assuming that not only were there bismuth atoms in the beam, but also some diatomic molecules, and perhaps even some molecules of Bi_8. The experiment also gave a heat of dissociation of Bi_2 molecules of 77,100 calories per mole. This is to be compared with the value of 56,000 calories obtained by Leu (151) in a careful magnetic deflection experiment.

Rabi and Cohen (194) and Ellett and Cohen (29, 41) have made use of the deflection of beams in a magnetic field to determine the velocity distribution. Stern and his colleagues (56) have studied the free fall of atoms in a beam. Here, too, the particles of different velocities undergo different deflections. This gives a very easy method of sorting velocities; however, the beam must be very long because the force of gravity is quite small, so that the intensity becomes very low.

Since the angle of diffraction of an atom at a crystal surface depends upon the de Broglie wave length, and therefore upon the velocity, the diffracted beam in any particular direction will be essentially monochromatic. As will be described later, this method of velocity selection was used by Estermann, Frisch, and Stern (52) in their study of the diffraction of helium at cleavage surfaces of lithium fluoride.

In every case, the results agreed fairly well with the predictions made from kinetic theory considerations. There were always some deviations which could be ascribed to scattering, finite wall thicknesses, and the like.

IV. SCATTERING OF MOLECULAR BEAMS

The molecular-beam method seems to be the only possible method for the study of the scattering of neutral particles by neutral particles. As was mentioned earlier, the first experiments of this type was performed by Born (14, 19), who showed roughly that the product of the mean free path and the pressure was a constant. The main features of the more recent investigations are the following: A narrow beam of a substance for which there is a quantitative detector is passed through a chamber containing a gas at controllable pressure. The shape and intensity of the scattered beam is compared with the shape and intensity of the parent beam for various scattering pressures. From these data the mean free paths and scattering cross sections may be determined.

The problem has been treated quantum-mechanically by Mizushima (180) and by Massey and Mohr (159). According to them the collision cross section for slowly moving hard spheres is two times the classical value if the particles are different, but four times the classical value if the particles are similar. If there are attractive forces between atoms, the newer theory gives a finite cross section, while the classical theory gives an infinite value. All of the investigators in this field—Knauer (130), Zabel (258), Eldridge (40), Fraser and Broadway (24, 69), Mais (154), and Rosin and Rabi (213)—have found cross sections much larger than those expected from the classical hard-sphere theory. Also Knauer (130), in his investigations of the scattering of helium by mercury, found that beams at high temperature are scattered more than beams at low temperature. These results are in disagreement with the older theory, but are to be expected on quantum-mechanical considerations. Mais (154) used an apparatus of sufficient resolution that particles deflected through 4.5 min. of arc were counted as scattered. He found that the scattering of potassium atoms by helium and by hydrogen agreed quite well with the quantum-mechanical predictions of Mizushima, Massey, and Mohr for hard spheres. However, for the cases of the scattering of potassium by neon, nitrogen, argon, and carbon dioxide, the collision radii were much larger than given by the theory, indicating considerable interatomic attraction. Rosin and Rabi (213), using a very refined apparatus with resolution of 1 min. of arc, studied the scattering of all the alkali atoms from the gases hydrogen, deuterium, helium, neon, and argon. The cross sections for potassium were in good agreement with those obtained by Mais, being in every case only a little higher. This indicates that the cross sections are really finite. Furthermore, the scattered beam had the same shape as the parent beam. These results all lead to the conclusion that the classical theory is entirely inadequate for the treatment of this particular problem of the scattering of a molecular beam.

Rabi (189) has stated that this leads to a new kinetic theory. The authors wish to point out, however, that for the majority of problems, such as the transport phenomena in gas (diffusion, heat conduction, and viscosity), the classical theory does give the correct result. There are a few exceptions at low temperatures. For these a quantum-mechanical kinetic theory must be used. It is even possible, with some assumptions, to obtain the van der Waals forces from scattering experiments. This was done by Fraser, Massey, and Mohr (72) for the scattering of potassium and sodium by mercury, using data obtained by Broadway (24). Torrey and Spremulli (247), who have just begun work on the problem, hope to do the same for the scattering of alkali atoms by alkali atoms.

Rosenberg (212) has compared the scattering of atoms and molecules of lithium and potassium by various inert gases. He separated the atoms from the molecules by deflecting them in a strong inhomogeneous magnetic field.[2] Although he has not published complete results, he has shown that the cross sections are very nearly the same for the two types of particles. For example, in the case of potassium, the ratio $\sigma_{\text{mol.}}/\sigma_{\text{atom}}$ varies from 1.20 to 1.36.

V. SURFACE PHENOMENA

As has been mentioned, many investigations have been made on the character and behavior of the deposit produced by the condensation of a molecular beam.

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[2] Compare Lewis, page 60.

Following Langmuir, it was assumed that, at least in the majority of cases, an atom striking a surface was adsorbed for a time and then reevaporated at random. Holst and Clausing (98) devised a molecular-beam method of measuring the time of adsorption, but were able to show only that it was very small. Veszi (251), with an improved apparatus, reflected a beam of heavy atoms from an olive oil surface moving rapidly at right angles to the beam. The last slit was in a large thin metal sheet kept cool with liquid air, and was just 2 mm. from the oil surface. Particles leaving this surface, either by reflection or reëmission after adsorption, were condensed on the cold plate. After taking into account the sidewise impulse given to the particles by the moving surface, Veszi found it possible to calculate the time of adsorption on the oil from the shape of the deposit.[3] For zinc and bismuth he found times of the order 10^{-5} to 10^{-4} sec.

Beeck (11) has determined the relative accommodation coefficients with respect to argon of various hydrocarbons at a nickel surface. He measured the sensitivity of a Pirani gauge used to detect the hydrocarbon beams as a function of the oven pressure, and extrapolated to zero oven pressure. By applying the Knudsen formula for the heat conducted by a gas at low pressure, both for the hydrocarbon with unknown specific heat and for argon, he was able to calculate the relative accommodation coefficients. By assuming that their actual value tends to unity for the more massive molecules, he was able to obtain absolute values for his whole series. This assumption also led to some information about the variations of specific heat with mass. As a check, it was found that the results for hydrogen were in good agreement with previous measurements.

The molecular-beam method is excellent for producing uniform thin films of an easily condensed material on a cool surface. It has therefore been used several times in the studies of such films. One can calculate the thickness of a layer laid down in a given time from a knowledge of the intensity of the beam if he assumes that the molecules are spaced as they are in the bulk. Mayer (162), following the previous work of Brady (20), investigated the photoelectric properties of thin films as a function of their thickness. He deposited a film of potassium on a platinum foil. In order to test the constancy of the beam over a period of time, he used the receiver as the filament of a Taylor surface-ionization gauge and measured the ionization current. Saturation current was reached when the foil was hot enough. Mayer was thus able to calculate the intensity of his beam by assuming that the efficiency of ionization was 100 per cent. He found that if he outgassed the potassium and the platinum carefully, the photoelectric emission stimulated by a quartz mercury lamp increased strictly linearly with the time until a little less than a monatomic layer had been deposited. After this, it increased less rapidly, but gave no evidence of a maximum. However, if the potassium was not carefully outgassed, the emission showed a maximum at the same thickness where the other curve broke. The secondary electron emission stimulation by electron bombardment was studied by Mayer in a similar manner. Johnson and Starkey (107) made measurements of the same type on the electrical conductivity of thin films of mercury. Starkey (234) has also studied the reflectivity of thin films of cadmium for visible light.

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[3] In this work it was found that cadmium and thallium were immediately reflected without being adsorbed. The deposits of these metals showed a remarkable structure. Around the central, approximately oval, spot were from six to eight rings showing a doublet structure, and entirely separated from each other. Veszi (252) has explained these as due to diffraction by the individual randomly oriented oil particles. He exhibited a photograph of a similar pattern produced by scattering light, with wave length 10^5 times the de Broglie wave length of the atoms, from lycopodium powder 10^6 times the size of the oil particles. This could not possibly be a Debye-Scherrer pattern, because the slit was long and narrow, so that the structure would be smeared out.

Copley and Phipps (31) have used the molecular-beam method to measure the surface ionization of potassium on tungsten as a function of temperature. For each temperature, they compared the ion current with that from an oxide-coated tungsten filament, for, according to Langmuir, the ionization is complete at that surface. They found a discrepancy with the theory which they explained by assuming that the work function varies with the temperature (205). Hendricks, Phipps, and Copley (96) have applied the same method to the study of the surface ionization of the potassium halides at an oxygen-free tungsten surface. For high temperatures (1800-2400°K.) the molecules were dissociated, the halogens leaving the wire as atoms, and part of the potassium being ionized. For lower temperatures, halogen layers appeared on the tungsten. Guthrie (87) has done similar experiments with barium. It has an ionization potential (5.16 volts) larger than that of tungsten (4.5 volts), and therefore should not be detected by a surface-ionization gauge. In disagreement with the theory, however, barium is ionized by clean tungsten. Guthrie estimated that the ionization efficiency was about 100 per cent if the temperature of the wire was 2000°K. or higher. It has been pointed out by Hay (95) that Becker, in some unpublished work, found efficiencies only about one-third to one-half as great. This is evidently a problem not as yet completely solved.

VI. DIFFRACTION AND REFLECTION OF MOLECULAR BEAMS

In the discussion of surface phenomena (page 247) it was shown that the time of adsorption for some atoms is extremely small or even zero. If the de Broglie postulate of the wave nature of matter is correct, atoms which are not adsorbed will be reflected *specularly*, provided the projections of the unevennesses of the reflecting surface upon the beam direction are less than the wave length. Any surface will give specular reflection if the glancing angle is small enough. The existence of this type of reflection is inferential proof of the wave nature of the beam particles.

In 1927, Knauer and Stern (133) obtained specular reflection of beams of molecular hydrogen and helium from highly polished metal surfaces at grazing angles of a few minutes of arc. However, they could find no evidence of diffraction at a grating ruled on speculum metal. These results were to be expected if the molecules had the de Broglie wave length. They soon announced the specular reflection of helium and hydrogen at large glancing angles of incidence from cleavage surfaces of sodium chloride crystals. Johnson (109) and Kerschbaum (129) showed the same for beams of atomic hydrogen.

Since hydrogen and helium have de Broglie wave lengths of the order of an Ångström unit, and since that is also the approximate lattice spacing of the alkali halide crystals, these latter should act as diffraction gratings for molecular beams. Certainly they will be *surface* gratings, not space gratings, for there can be no penetration of the particles into the crystal. Since the crystals are of the cubic type, they will act as two-dimensional, or crossed, gratings with the principal axes at right angles to each other. If the incident beam makes angles α_0 and β_0 with these axes, and the diffracted beam angles α and β with the same two directions, then

$$\cos\alpha - \cos\alpha_0 = h_1(\lambda/d)$$
$$\cos\beta - \cos\beta_0 = h_2(\lambda/d)$$

where d is the grating constant and h_1 and h_2 are integers, the orders of diffraction. These equations are, of course, just the ordinary grating law applied twice. They are the equations of cones the axes of which are the rulings on the grating at the point of incidence of the beam. There will be diffraction mixima where two cones intersect. If one put a sensitive plate in a position to receive the reflected beam of atomic hydrogen, he might expect to find a spot representing the specularly reflected beam, and symmetrically placed around it other spots corresponding to the various orders of diffraction. Unfortunately, this will not really occur, for, as was mentioned previously, the particles in the beam have a wide range of velocities and therefore of wave lengths. Since each wave length is diffracted through a different angle, each spot will be spread out into a broad band.

Stern and Knauer (133) looked for evidences of the diffraction of hydrogen molecules and helium atoms at cleavage surfaces of sodium chloride. They used as a detector a hot-wire manometer so arranged as to receive successively all the diffracted atoms of order (0, ±1). Faint evidences of maxima were found in the expected place (239). When lithium fluoride was substituted for the rock salt, in a later experiment of Estermann and Stern (57), absolute proof of the existence of the diffraction of atoms was obtained. The maxima were found exactly where one would expect them if the velocity distribution is taken into consideration. This was true for both hydrogen and helium. Further, the maxima were shifted in the proper directions and by the proper amount when the temperature of the incident particles was changed. The grating rulings are the rows of like ions, and the grating space which must be used in the calculations is the distance between these rows. Thus this constitutes a direct experimental proof of the validity of the de Broglie relation for heavy particles. Similar results were found for atomic hydrogen by Johnson (110, 111).

Not satisfied with this, Estermann, Frisch, and Stern (52) studied the reflection and diffraction of monochromatic beams. These were produced either by a mechanical velocity sorter or by the use of a double-crystal spectrometer. Using a rather crude mechanical velocity sorter, Stern and his coworkers in Hamburg proved the correctness of the wave picture of matter and verified the de Broglie relation between wave length and velocity. This simple experiment is thought to be one of the most beautiful and direct demonstrations in modern physics.

With the double-crystal spectrometer, the atoms diffracted in a particular direction from the first crystal have only a small range of wave lengths and therefore of velocities. If these are reflected again, the resulting pattern will be much more highly resolved than with only a single crystal. Again de Broglie's expression for the wave length was valid to within the experimental error of about 1 per cent. Incidentally, these experiments also gave a very good proof of the Maxwell law for the distribution of velocities.

In their further investigations, both Stern and his collaborators and Johnson found some unexpected results. The latter (111, 112), who worked with atomic hydrogen and detected it with a molybdenum oxide plate, found faint traces of diffracted beams very close to the specularly reflected beam. He assumed that they were due to a secondary lattice of the type postulated by Zwicky. This lattice spacing was estimated to be 100 Å. or more. However, even with high resolution and almost grazing incidence, he could not separate these secondary maxima from the specularly reflected beam. This led him to make the suggestion (113) that the spacing in the secondary lattice is not constant.

Frisch and Stern (76) found that with high resolution the diffraction patterns were not smooth curves, as expected, but had reproducible minima. Similar effects were found in the curves of reflectivity as a function of the orientation of the crystal. Frisch (73) summarized these results as follows: An atom will not be specularly reflected whenever a certain very simple (but empirical) relationship exists between the two components of its incident momentum perpendicular to the surface and parallel to one of the lattice directions. Furthermore, an atom will not be diffracted in such a direction that the same relation would obtain between the components of its momentum as it leaves the crystal surface.

These results were given a theoretical explanation by Lennard-Jones and Devonshire (33, 149). They showed that under certain conditions it is possible for an atom to be adsorbed by the crystal without change of kinetic energy, instead of being diffracted. The conditions necessary for this selective adsorption had the same form as those found empirically by Frisch. From a comparison of theory and experiment, Lennard-Jones and Devonshire were able to calculate the energies of adsorption of helium at a lithium fluoride surface. These investigators and Lenz (150) and Brandt (21) have calculated the intensities of various orders of diffraction maxima; however, no one has as yet performed a sufficiently precise experiment on the higher orders which would suffice to substantiate or contradict their results.

Stern and Frisch (76) also found that the reflectivity of lithium fluoride for helium differed slightly when they used differently shaped slits. They suggested that this also might be due to other beams very close to the specularly reflected beam, such as could be produced by diffraction from a secondary lattice. To test this latter hypothesis, Bessey (13) investigated the specularly reflected beam very carefully, but could find no evidence of a secondary diffraction maximum. He found, however, that the specularly reflected beam had a fine structure which varied from crystal to crystal and from place to place on the same crystal. This could not be a diffraction phenomenon, since the reflection patterns were identical for beams of helium and molecular hydrogen, although the wave lengths were different. The simplest explanation is that the crystals, although supposedly single, are actually made up of many tiny crystallites tipped at small angles (a degree or less) with respect to each other. Such a crystal structure might be responsible for the effect found by Johnson. This explanation would seem to confirm the work of Renninger (64, 206), who was led to essentially the same hypothesis during his study of the reflectivity of rock salt for very narrow beams of x-rays.

Diffraction curves have been definitely found only for helium and for both atomic and molecular hydrogen. Best results are obtained with lithium fluoride. Zabel (257) showed that with extremely great precautions good results can be obtained with rock salt; however, since it is so hygroscopic, the crystal surface is soon spoiled. In fact, when the crystal is exposed to water vapor, the diffraction pattern changes and various anomalous results are obtained. Zabel tried experiments with neon and argon also, but the results were inconclusive.

It is not to be expected that diffraction patterns would be found for metal beams, for the wave lengths are much smaller than those of hydrogen and helium, and are therefore small compared to the lattice spacing. More important is the fact that all metal atoms have a tendency to be adsorbed. The results which are found agree in certain cases with the cosine law of reflection (255). For example, Taylor (244) reflected beams of potassium, lithium, and cesium from sodium chloride and lithium fluoride over a wide range of angles of incidence. Although he could have detected specular reflection present to one part in 10,000, he found no trace of it; the angular distribution of the reflected beam followed the cosine law exactly. Ellett and Cohen (42) found the cosine law to hold for potassium atoms reflected from magnesium oxide. They made a velocity analysis of the reflected beam and found the distribution to be the expected one, that corresponding to the temperature of the crystal and not of the incident beam. In this case, at least, the mechanism is indeed the one postulated by Langmuir, adsorption and consequent reëvaporation.

However, in some investigations, intense reflection was found in approximately the direction of the specularly reflected beam (44, 45, 46, 47, 90, 114, 119, 261, 262). Although there is not complete agreement between the various results, it seems that when the crystal surface is carefully prepared and when the diffuse background (cosine-law scattering) is subtracted from the total reflection, there remains a beam the direction of which does not deviate very much from that to be expected for specular reflection. However, this beam is very much wider than would be expected from considerations of the geometry involved.

VII. MAGNETIC DEFLECTION

A. Theory

According to theory, every system which has a single angular momentum vector j (measured in units of $h/2\pi$) has also a magnetic moment $\mu = gj$, where μ is measured in units of the Bohr magneton $\mu_0 = eh/4\pi Mc$, and g is the gyromagnetic ratio in units of $e/2Mc$. Here M is the mass of the electron. Often a unit called the nuclear magneton is used; it is defined in the same way, except that M is in that case the mass of the proton. The quantum number j may have integral or half-integral values. If this system is placed in a magnetic field, its behavior can be explained by assuming that its angular momentum vector precesses about the field direction with frequency $\nu = gH(e/2Mc)$. This is the well-known Larmor precession. The projection of the angular momentum j upon the field direction will have only the $2j + 1$ values $\pm j$, $\pm(j-1)$, $\pm(j-2)$, and so on, each designated by quantum number m_j. The energy of the system will be changed by an amount $\Delta E = m_j h\nu$. A beam of atoms will thus be split in a magnetic field into $2j + 1$ parts, each of which is in a separate energy state. We say such a beam has been space-quantized.

If a system has more than one angular momentum vector (such as electron orbital momentum, electron spins, nuclear spins, and molecular rotation), the magnetic moment associated with each one will interact with all the rest. This interaction is called coupling. When such a system is placed in a magnetic field, the resulting motion of the momentum vector is very complex. However, if the energy changes produced by the field are large compared to those due to the coupling, that is, if the field is strong enough, the individual angular momentum vectors will precess independently about the field direction. In fields which are not strong, one may use the perturbation method to calculate the energy changes. In any case the force in a certain direction on a system in a magnetic field is equal to the directional derivative of the energy change produced by the field.

In the following, we shall use the usual notation: in units of $h/2\pi$, j is the total extranuclear angular momentum, i is the nuclear spin, and f is the resultant of the two. The projections of each of these on the field direction are denoted m_j, m_i, and m.

B. Electronic moments

After the power of the molecular-beam method for the measurement of the magnetic properties of atoms and molecules had been demonstrated by Stern and Gerlach (80), many investigations of magnetic moments were made. Although in recent years the emphasis has been on nuclear measurements, there have been several important studies of electronic moments.

In 1933, Meissner and Scheffers (165), at the Reichsanstalt in Berlin, made a determination of the atomic moments of lithium and potassium. With the wedge-slot field such as was used by Stern (151) in Hamburg, the results for both materials were identical, i.e., $(0.916 \pm 0.005) \times 10^{-20}$ C.G.S. units for the Bohr magneton. The limit of error seems to be a bit optimistic, in view of the large amount of scattering of the beam which would distort the Maxwell velocity distribution.

In 1938, Ramsey and Kellogg (127) passed beams of helium, argon, and neon through magnetic fields with extremely high inhomogeneities. As was to be expected, these atoms exhibited no magnetic property beyond that attributable to their diamagnetism.

Certain molecules have an electronic magnetic moment; examples are oxygen and nitric oxide. Van Vleck (249) has worked out the theory of space quantization for these molecules. The problem was attacked experimentally by Simpson (232) and Schnurmann (224) independently. They found that the beam behaved qualitatively as expected, but that there was a strong interaction between the rotation and the electronic angular momentum. Their results were similar, indicating that the prediction of Van Vleck would not be valid until much higher fields were obtained.

Recently, Stern (56, 240) and his colleagues in the Molecular Physics Research Laboratory began working on a new precision method of measuring the Bohr magneton. In principle, it is very simple; it consists merely in balancing the gravitational force on a very long beam by an upwards magnetic force (produced by the inhomogeneous field surrounding a conductor of electric current). Thus

$$N\mu \frac{\partial H}{\partial X} = Nmg$$

where N is Avogadro's number, m is the mass of the atom, and g is the acceleration of gravity. Since g, Nm, and $\partial H/\partial X$ are well known, it should be possible to obtain a very precise value of the molar magneton $N\mu = Neh/4\pi mc$. $F = Ne$ and c are known very accurately; therefore we have here a method of obtaining h/m precisely. This general method of the molecular balance should be very powerful. For instance, Stern (241) has suggested that one could get complete separation of isotopes by balancing a magnetic force against a centrifugal force.

C. Requantization

It is of interest to inquire as to the effect on a space-quantized beam of passing it through a magnetic field. Will requantization take place, or will the atoms remain in the same states? The first experiments on this problem were performed by Stern and his collaborators (75, 186) in Hamburg. They selected one component of a beam which had been split in a Stern-Gerlach apparatus, passed it through a field which changed direction along the path of the beam, and analyzed it in a second inhomogeneous field. When more than one maximum was found, it was obvious that requantization had occurred.

Special cases of this problem have been treated theoretically by Güttinger (88), Majorana (155), and others (181, 192, 211, 228). They showed that if the transition is adiabatic, the atom which was precessing in the first field will precess in the same way in the second field. However, in the case of non-adiabatic transitions, that is, in case the time during which the field changes direction is of the same order of magnitude or less than the time for the Larmor precession, there will be a reorientation of some of the atoms. In the simple case of the alkalies, where only two states are possible, these reoriented atoms may be said to have "turned over"[4] from the original state to the other one. The results of the experiments in general agreed with the theory.

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[4] Because these "turning over" experiments were first performed by Phipps, they are known familiarly as the Phipps flops.

At present Winslow, in Stern's laboratory, is continuing experiments to examine the effect, if any, of the presence of paramagnetic scattering gases on the amount of reorientation. Rabi and his colleagues have used non-adiabatic transitions very effectively in their recent measurements of nuclear magnetic moments.

D. Nuclear moments

Stern (238) had already predicted in 1926 that the molecular-beam method could be applied to the measurement of nuclear magnetic moments. Since these have magnitudes about 1/2000 those of the electronic moments, the problem is, of course, very difficult. If substances with the latter are used, the deflections due to the nuclear moment will be only of the order of 0.1 per cent of the total deflection and will not be easily observable. One method of avoiding this is to measure the very small deflections of molecules with zero electronic moment. This method was applied to hydrogen by Estermann, Frisch, and Stern (53, 58, 60, 61, 62, 77). The total nuclear moment of a hydrogen molecule will be either zero or twice that of the proton, depending on whether it is of the para or ortho variety. The experiments were performed with hydrogen at the temperature of liquid nitrogen in order to reduce the complications due to the presence of rotational magnetic moments. These are of the same order of magnitude as the nuclear moments and are obtained to a sufficient degree of approximation from the deflection of pure para-hydrogen in the higher rotational states and a knowledge of the population of these states. The results of the first measurements were very surprising; the moment of the proton was two and one-half times as great as expected.

These experiments were continued and refined in this country. In the final work, besides beams of ortho-hydrogen, beams of HD were used (54), because the populations of the higher rotational states are small. The proton moment could be calculated from the ratio of the beam intensities at the undeflected position when the field was on and when it was off. This calculation depends upon a knowledge of the geometry of the slit system, the validity of the Maxwellian velocity distribution, the value of the inhomogeneity of the field, and the approximate values of the deuteron and rotational magnetic moments. To eliminate any deviations from the assumed velocity distribution, measurements were taken for various residual scattering pressures and extrapolated to zero pressure. The inhomogeneity was measured by three methods: the variation of the resistance of a bismuth wire with field strength, the force on a piece of bismuth suspended in the field by a quartz fiber, and by the use of a flip-coil. The values of the deuteron and rotational moments come in only as small corrections and therefore do not need to be known very precisely. As their final result, Estermann, Simpson, and Stern (54, 55) obtained for the proton moment the value 2.46 ± 3 per cent nuclear magnetons; the deuteron and rotational moments are roughly one-third as great.

In 1931, Breit and Rabi (23) published a molecular-beam method of determining both the nuclear spin and the hyperfine structure separation. From a knowledge of these two quantities, one may calculate the nuclear magnetic moment by using equations of Goudsmit (83) and Fermi and Segré (65). However, these calculations depend upon the evaluation of wave functions at the nucleus, and are therefore only approximate except in the case of the hydrogen atom, the wave function of which is exactly known. The method is based on a deflection in a field sufficiently weak that the electronic and nuclear spins are still coupled to some extent; that is, the electronic angular momentum and the nuclear angular momentum are not quantized separately, and therefore do not precess about the field direction independently of one another.

Rabi (197, 198, 202) first applied this method to the hydrogen isotopes. In these measurements he used beams of atoms, deflecting them in a weak magnetic field and detecting them with a molybdenum oxide plate. He obtained in this way a value of the proton moment of 3.25 ± 10 per cent nuclear magnetons, thus confirming the extremely high value of Stern. This experiment can not be considered as anything more than preliminary work. However, it has one notable feature: the weak magnetic field was produced without iron, by passing very large electric currents through two long water-cooled copper tubes placed parallel to the beam path. The field was thus absolutely calculable.

Rabi (28) soon introduced a refinement into his experimental method which simplified the calculations considerably and which made the results independent of the velocity distribution in the beam. Consider atoms with electronic angular momentum 1/2 and nuclear spin i. Then there will be two possible states with total angular momentum $f = i + 1/2$ and $f = i - 1/2$. These states will be separated by an energy $\Delta W = hc\Delta\nu$, where $\Delta\nu$ is the hyperfine structure separation which can be obtained spectroscopically in certain cases.[5] If now the atoms are placed in a magnetic field, their energies will be changed and each of the two states will be split into $2f + 1$ separate levels of equal probability and designated by total magnetic quantum number m. The atoms in each of these states will have effective moments μ_m (in units of the Bohr magneton μ_0) which depend upon the amount of coupling between the nuclear and electronic spins, that is to say, upon the field strength H. The effective moment is given by the formula

$$\mu_m = \pm \frac{2m/(2i + 1) + x}{[1 + 4mx/(2i + 1) + x^2]^{1/2}} \mu_0$$

Here x is equal to $(2\mu_0 H)/(hc\Delta\nu)$. In this formula the nuclear moment has been neglected with respect to the electronic moment. The different signs refer to the two original degenerate f states. If the nuclear magnetic moment is in the same direction as the nuclear spin (positive moment), then the positive sign refers to the state $f = i - 1/2$. Figures 1 and 2 show plots of μ_m/μ_0 against x for the cases $i = 6/2$ and $i = 7/2$ under the assumption that the nuclear moment is positive. The dotted lines refer to the $2f+1$ states for $f = i - 1/2$. If the nuclear moment is negative, the figure will be exactly the same, except that the effective moment corresponding to each state will be reversed in sign.

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[5] Here we see the superiority of the molecular-beam method. If $\Delta\nu$ is very small, it is impossible to resolve the lines of the spectrum. However, as Stern has pointed out, it is always possible, by making a molecular beam long enough, to resolve two components which suffer different deflections. We can see this by applying the uncertainty principle in the form $\Delta W \Delta t \cong h$. If the particles have an average velocity v, then the time of flight through the distance L is L/v; therefore the length L necessary for separation will be of the order $(hv)/W$.

As can readily be seen, for certain values of x, that is, for certain values of H, some of the states will have effective moments equal to zero. This is true when

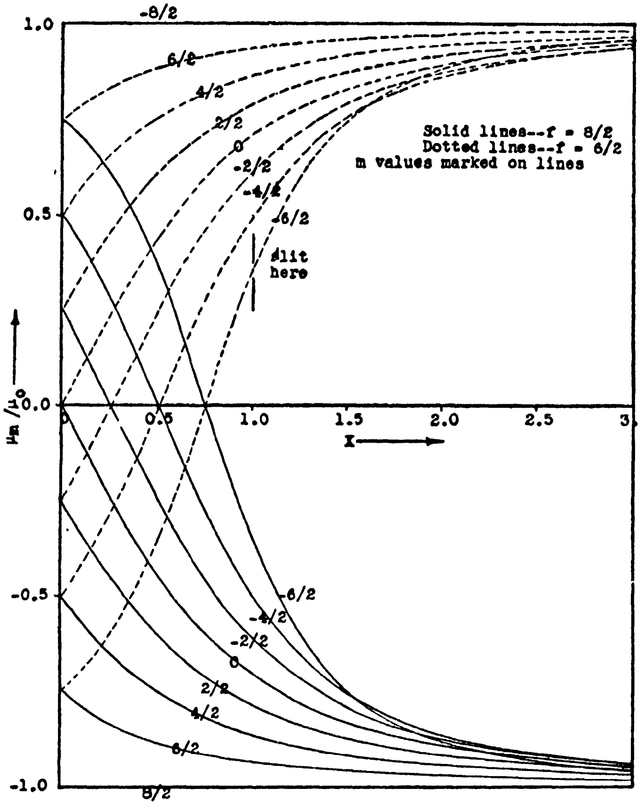

FIG. 1. Effective magnetic moment *versus* field strength. Nuclear moment assumed positive. Case 1: $i = 7/2$.

$x = (-2m)/(2i + 1)$. If the magnetic field is inhomogeneous, all the atoms except those with zero moment will be deflected. The number of different values of x for which this can occur depends upon the value of i. For the cases shown in the figures there are three such zero moments; for the cases $i = 4/2$ and $i = 5/2$ there are two, and so on. From the determination of the relative x values (relative H values) and the number of zero moments it is possible to specify the spin exactly. It is then a simple matter to determine the absolute value of x for one of the states, and therefore the hyperfine structure separation.

Experiments of this type were performed by Rabi and his colleagues (67, 166).

The resolving power of the apparatus was very high; the atoms with non-zero moments were deflected enough so that they missed the detector completely. Values of the nuclear magnetic moments were obtained by means of the Goudsmit–Fermi–Segré formulas (65, 83). All the alkali metals, atomic hydrogen and deuterium, indium, and gallium were studied in this way. For the cases in which the nuclear spin was 3/2 or less, the actual procedure was a little more complicated than indicated above, but the principle was essentially the same.

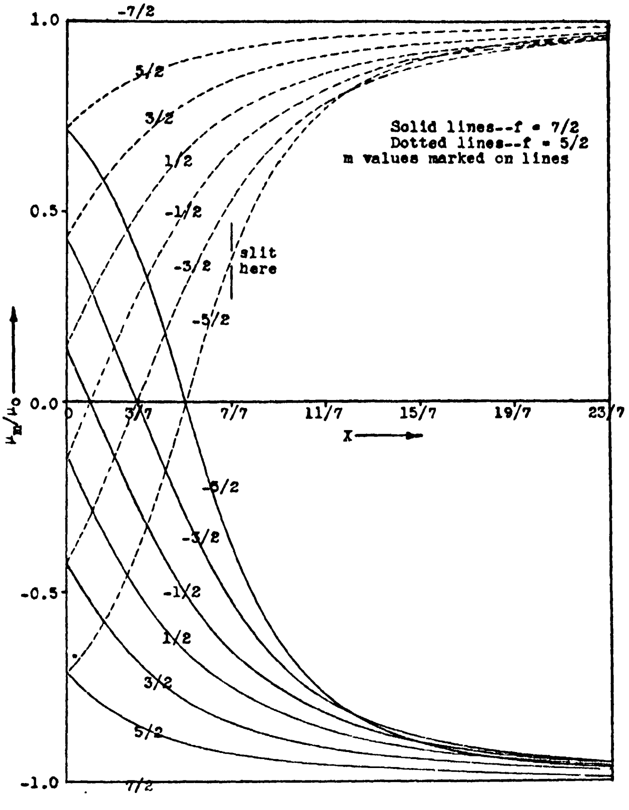

FIG. 2. Effective magnetic moment *versus* field strength. Nuclear moment assumed positive. Case 2: $i = 6/2$.

The results in general agreed with the spectroscopic values, where these were known. The case of hydrogen is of especial interest; the proton moment was found to be 2.85 nuclear magnetons, a value considerably higher than that found by Estermann, Simpson, and Stern. This method is applicable to mixtures as well as to pure substances and has, therefore, been used to measure the moments of isotopes without separating them (190).

Two important extensions of this method were made. The first (178, 191) permits a determination of the sign of the nuclear magnetic moment. As was pointed out previously, if a beam of atoms is passed through an inhomogeneous magnetic field, it is split into a number of overlapping beams, the Zeeman components of the two original states. It can easily be seen from the figures that, if the field is very strong, the deflection pattern will have two components, corresponding to the two possible orientations of the electronic moment. However, if the field is rather weak, the different components will all have different deflections. For simplicity, let us consider a field of such a magnitude that the parameter x has a value about equal to unity. In this case, all the atoms in the state $f = i - 1/2$ have effective moments of the same sign, and will therefore be deflected in the same direction. All the atoms in the state $f = i + 1/2$ except those for which $m = -f$ are deflected in the opposite direction. We do not, of course, know which group of atoms has the positive effective moment. Let us choose one of the components for which $m = -(f-1)$ by means of a selector slit and pass it through a weak field varying non-adiabatically. Transitions will occur, subject to the selection rule $\Delta f = 0$ (181). Now pass the beam through such a strong field that there is complete uncoupling and the effective moment is only $\pm\,\mu_0$. If we had picked a component of the state with the smaller value of f, corresponding to a dotted line in the figure, we would now find only one maximum in the deflection pattern. This is because all the components of this state had originally a deflection in the same direction, and transitions can not occur which give deflections in the opposite direction. However, if we had picked a component of the state with $f = i + 1/2$, corresponding to a solid line in the figure, we would find two maxima in the deflection pattern, because some of the transitions would have been to the state with $m = -f$, which had an effective moment and consequently a deflection of the opposite sign. From a knowledge of the quantum number f and the sign of the atomic moment, one can tell the sign of the nuclear moment. Rabi and his collaborators showed that all the nuclear moments studied by this method were positive.

The second important extension (192) of the zero moment method was to increase the resolution to such a point that the nuclear magnetic moment was no longer negligible with respect to the electronic moment. In this case the nuclear moment can be determined directly. Millman, Rabi, and Zacharias (176) studied beams of indium in this way; detection was with a Taylor surface-ionization gauge using an oxide-coated filament. They found it necessary to use iron fields again; the fields produced by the long wires parallel to the beam did not give large enough deflections. However, instead of using the bulky magnet with the wedge–slot pole pieces of Stern and Gerlach, they used one which was small enough to go into the vacuum system, was easily constructed, and for which it was not difficult to calculate the inhomogeneities. This consisted of a long piece of Armco iron tubing with a slot cut in it lengthwise; in the slot were fitted pole pieces of simple design. The iron was magnetized by passing large currents through four turns of water-cooled copper tubing.

Zero moment peaks were found, not only for the normal $2p_{1/2}$ state, but also for the metastable $2p_{3/2}$ state. It was suggested that careful examination and location of these peaks would give information on the interval rule and the magnitude of the nuclear quadrupole moment. This latter was actually measured in this way by Hamilton (89); later Renzetti (207) did the same thing for gallium. It is interesting to note that Stern had predicted such measurements twelve years before (238).

In 1937 Rabi (193) published the theory for a more elegant method of determining both the sign and the magnitude of the nuclear magnetic moment in cases where the nuclear spin is known (16, 26, 242, 246). This method is absolutely independent of the velocity distribution in the beam, and necessitates only measurements of frequencies in the radiofrequency range and magnetic field strengths. It is relatively insensitive to nuclear transitions in systems with electronic angular momentum, and therefore has usually been used to study beams of molecules with zero electronic moment. However, extensions have been made to atomic beams; from a study of these it is possible to learn something about their spectra in the radiofrequency range.

Suppose a molecule with zero electronic moment is placed in a magnetic field H_0 which is strong enough to uncouple all moments of nuclear magnitude but which is too weak to uncouple the electronic spins. The original energy state represented by the nuclear quantum number i will be split in the field into $(2i + 1)$ states, each designated by a quantum number m. This means that the angle of precession between i and H_0 will be different for the atoms in different states, and therefore the effective moments will be different. If now we apply, perpendicular to the original field, an additional small magnetic field H_1 which rotates with the frequency f, the probability can be calculated for transitions between states m_1 and m_2. This probability is very small, except when the direction of rotation is the same as the direction of precession and the frequency is very close to the Larmor frequency ν; the maximum occurs when $f = \nu$. The frequencies necessary are of the order of a few kilocycles.

Rabi's method is very simple indeed. A beam is sent through a strong field H_0 with a superimposed weak rotating field H_1 and a combination of field strength H_0 and frequency is found which produces the largest number of transitions. Then this frequency is the Larmor frequency corresponding to the magnetic moment being studied. From a knowledge of the field, the frequency, and the angular momentum, the moment can easily be calculated. The direction of rotation of the weak field gives immediately the direction of the precession and therefore the sign of the magnetic moment.

In order to put this method into practice, Rabi gave up the rotating field and used an oscillating one instead. The frequency of oscillation plays the same rôle as the frequency of rotation in the theory outlined above. The experimental details are as follows (201): The molecular beam passes through a strong inhomogeneous magnetic field and is therefore deflected. The individual beam particles have different effective moments and different velocities; therefore the deflections will all be different. The beam now passes through a strong homogeneous field and into a second inhomogeneous field exactly like the first, except that its inhomogeneity is in the opposite direction. Thus all the particles will be given a deflection equal and opposite to the first, so that the beam intensity at the receiver is the same as when the fields are off entirely. Now a small oscillating field of variable frequency is applied perpendicular to the middle homogeneous field; obviously, if transitions occur, those molecules undergoing such transitions will have different effective moments than before, and therefore will not be refocussed at the detector by the second field. Thus there will be a decrease in the intensity recorded by the detector. The frequency is changed until this decrease is a maximum; that is, until the beam intensity is a minimum. When this occurs, the frequency is equal to that of the Larmor precession. It is essential for the success of the experiment that the three fields be connected together with iron. Otherwise, there is a danger that the change from field to field would be so sudden as to induce non-adiabatic transitions, which would invalidate the results of the experiment.

Rabi thought at first that by using the oscillating field he was giving up the ability to determine the sign of the nuclear moments, because the oscillating field is perfectly symmetric. This field was produced by passing an alternating current through a hairpin-shaped piece of wire. Millman (167) pointed out, however, that as a molecule enters and leaves this field, the end effects are exactly the same as if the field were rotating. These end effects lead to an assymmetry in the intensity–frequency curve which depends upon the sign of the moment. (Actually, the prediction is for a second minimum, but the resolution is insufficient to obtain it.) From this assymmetry the sign can easily be determined.

The first application of this method was to the alkali metals (200, 203), since the Taylor gauge is such a sensitive detector for these materials. The first moments measured were those of Li^6, Li^7, and F^{19}; these were used in the molecules lithium chloride, lithium fluoride, Li_2, and sodium fluoride. In every case the values of f/H_0 for the observed minima were constant over wide ranges of the magnetic field. This indicates that they are due to nuclear and not molecular transitions; the latter would not possess a frequency proportional to the field. Two of the minima observed for the three molecules containing lithium were the same for all three molecules; these must certainly correspond to the nuclear moments of the two lithium isotopes. The deeper one was ascribed to the more abundant variety. There was also a common resonance minimum for the two molecules containing fluorine; this must of course be due to F^{19}.

Resonance minima are never ascribed to nuclei unless they are found in at least two different molecules. Then, if the spins are known, the nuclear moments are easily calculated. Since the time of the first publication of this magnetic resonance method, Rabi and his colleagues have applied it to a great many nuclei, usually in molecules containing an alkali atom, because of the ease of detection.

The one big exception is in the study of hydrogen. In this case gaseous hydrogen was used, and detection was by means of the Pirani gauge. Furthermore, because of the fundamental nature of the problem and because of the discrepancy between the previously obtained results, Rabi made a much more detailed investigation of the hydrogen molecules than of any others (125, 126).

The calibration of the apparatus was checked by using dichlorodifluoromethane; the value obtained for the nuclear moment of F^{19}, 2.623 nuclear magnetons, agreed well with the value 2.622 obtained with the previously used apparatus and a surface-ionization gauge.

The hydrogen experiments were made with H_2, HD, and D_2, all at the temperature of liquid nitrogen. At this low temperature the majority of the molecules are in rotational states with quantum number j equal to 0 or 1; the higher states are so thinly populated that they can be neglected. H_2 will be made up of molecules of para-hydrogen with nuclear spin $i = 0$ and rotational angular momentum $j = 0$, and ortho-hydrogen with $i = 1$ and $j = 1$. D_2 will consist of three types of molecules with the following (i, j) values: ortho-deuterium, (0, 0) and (2, 0), and para-deuterium (1, 1). HD molecules can have any combination of spin and rotational angular momentum. The molecules with zero spin and zero rotational momentum are not deflected in the magnetic field, and consequently behave as inert gases. Thus no results can be obtained from para-hydrogen, or from the ortho-deuterium for which $i = 0$. The ortho-deuterium molecules for which $i = 2$ will behave similarly to alkali molecules. Transitions can occur in either direction between the various m states; the frequencies and field strengths corresponding to these transitions give the deuteron moment. Similar measurements can be made with HD in the zero rotational state; from these may be obtained values of both the proton and the deuteron moments. The results are as follows: $\mu_H = 2.785 \pm 0.02$ nuclear magnetons, $\mu_D = 0.855 \pm 0.006$ magnetons. The former value agrees with the previous results of Rabi but disagrees with that of Stern.

To complement these results, Rabi made further measurements of the magnetic resonance spectra of molecules not in the lowest rotational state. In this case there is an interaction between the spins and the rotation so that the simple theory no longer holds; transitions will not occur when the applied frequency is exactly the Larmor frequency, but at small deviations from it.

Rabi solved the wave equation for the rotating hydrogen molecule in the magnetic field, including in the Hamiltonian the interaction of the nuclear spins with the applied field, of the rotational moment with the applied field, of each nuclear spin with the field produced by the rotation of the molecule, and the mutual interaction of the two nuclear moments. For the case of ortho-hydrogen in the first rotational state, the energy levels can be expressed in terms of two parameters, H', the field at a proton due to the rotation, and $H'' = \mu_H/r^3$, the field at one proton due to the magnetic moment of the other. Here r is the internuclear distance. In case the energy term due to the interaction with the external field is large compared to all the other terms, as was true for the experimental arrangement, there are nine levels. These correspond to the three orientations of the nuclear spin for each of which three orientations of the rotational moment are possible.

In performing the experiment the frequency f was kept constant and the magnetic field varied. Whenever the field had such a value that the Bohr relation $hf = E_n - E_m$ was satisfied, transitions would occur, accompanied by the absorption or emission of radiofrequency radiation. There are rigorous selection rules: $\Delta m_i = \pm 1$ or $\Delta m_j = \pm 1$, where m_i and m_j are the projections of i and j in the field direction. These give rise to two groups of six lines each, one located at approximately the field such that the applied frequency is equal to the Larmor frequency of the proton moment, the other at approximately the field such that the applied frequency is the Larmor frequency of the rotational moment. For the time being, we shall consider only the former group.

All six lines (minima in the resonance spectrum) were found experimentally. From the changes in the assymmetry of the pattern with frequency, it was possible to assign the appropriate quantum numbers to each line. Then from the theory and the position of the lines it was a simple matter to calculate the constants H' and H''. Completely consistent results were obtained, thus indicating that the theory must be essentially correct. To make a real check, the value of $H'' = \mu_H/r^3$ was multiplied by the average cube of the separation of the nuclei, obtained by Nordsieck from band spectra, to give a value of μ_H identical with that obtained directly from the Larmor frequency.

The theory which worked so beautifully for the gas ortho-hydrogen should also apply to para-deuterium, for everything is the same,—spin, rotational angular momentum, and internuclear distance. It is easy to calculate the effect of the change of mass on the spacing of the lines. However, the prediction does not agree with experiment; there are six lines as expected, but they are six times as far apart as they should be.

Rabi explained this discrepancy by assuming that the deuteron has an electric quadrupole moment, the reaction of which with the gradient of the molecular electric field tends to increase the separation. This interaction can be represented by a parameter H''' (in gauss) $= -(5e^2qQ)/(4\mu_D)$, where Q is the quadrupole moment, q is defined by $qe = \frac{\partial^2 V}{\partial z^2}$, e is the electronic charge, and V is the electrostatic potential at the nucleus. It turns out that the quadrupole interaction is of the same spin–spin type as that between the two deuteron moments. Therefore, the theory applied to H_2 will apply here if the quantity H''_H is replaced by $(H'' + H''')_D$. As indicated above, one can calculate from the proton value what H'' should be, and subtract it from the measured value to obtain H'''.

It is possible to obtain H''' directly from similar experiments on HD in the first rotational state. Here there are two groups of lines, one of nine components near the position of the Larmor frequency of the proton, the other of twelve components near that of the deuteron. The analysis is quite similar to that discussed above.

From the experimental value of the quadrupole interaction constant and the theoretical value of the field gradient as calculated by Nordsieck (183), Rabi computed the quadrupole moment and found that it was 2.73×10^{-27} cm.^{2}, good to about 2 per cent. In every case the values of the proton and deuteron magnetic moments agreed with the previous results.

Ramsey (204) performed similar experiments with the hydrogens, studying the groups of lines near the Larmor frequencies of the rotational moments. He outlined a theory showing that the rotational moment is proportional to the reduced mass of the molecule. This means that the moments of H_2, HD, and

D_2 should vary as 4:3:2. The experimental values for the moments in the first rotational state are 0.8787 ± 0.0070, 0.6601 ± 0.0050, and 0.4406 ± 0.0030 nuclear magnetons, respectively. The value for H_2 agrees with the experimental value of Estermann and Stern (53, 58) and with the theoretical values of Wick (253) and Brooks (25). A further experiment with para-hydrogen in the state $j = 2$ gave the moment $1.757 \pm 0.014 = 2 \times 0.8787$ nuclear magnetons, as expected. It will be noticed that there is a slight discrepancy in the 4:3:2 ratio. It was persistent, and always in the direction of smaller moments for the lighter molecules. This might indicate an effect of the zero state vibrational motion upon the mean separation of the nuclei.

Ramsey's analysis of the fine structure of the spectra confirmed completely all of Rabi's previous work on the interaction constants of H and D. He carried the interpretation still further, and deduced the high-frequency contribution to the diamagnetic susceptibility of H_2, and the dependence of this susceptibility upon the orientation.

Recently Rabi and his colleagues (144, 145, 172) extended the magnetic resonance method to the study of the radiofrequency spectra of *atoms*, in order to determine the hyperfine structure separations. Most of the experimentally observed hyperfine structures of atomic ground states lie in the radiofrequency range. The life-times of these energy states are very long, and consequently the intensity of spontaneous emission is low. Therefore, direct optical observation of the radiation is difficult. However, if an atom is irradiated with electromagnetic radiation of the proper frequency and intensity, it will either absorb or emit a quantum of this frequency. This change of state will cause a reorientation of the atom in a magnetic field, and can therefore be detected by the magnetic-resonance method. This gives extremely high resolution; thus it is superior to optical methods and to the method of zero moments.

In performing experiments of this type two cases must be considered: In the first, the central homogeneous field is very weak; therefore the energy states of atoms with angular momentum f are split into $2f + 1$ levels. Transitions will occur subject to the selection rules $\Delta f = 0, \pm 1$; $\Delta m = 0, \pm 1$. For weak enough fields, the center of gravity of the spectral pattern is the hyperfine structure separation $\Delta\nu$. In the second case the middle field is very strong. Here transitions give rise to lines the frequencies of which are independent of the field strength, and approach the limit $\Delta\nu/(2i + 1)$. This enables measurements to be made at a more convenient range of frequencies. Both cases lead to the same results.

For example, in the case of K^{39} the Zeeman pattern was well resolved at a magnetic field of 0.05 gauss. The hyperfine structure separation determined in weak fields is $(461.75 \pm 0.02) \times 10^6$ sec.$^{-1}$, or 0.015403 cm.$^{-1}$; that measured in strong fields (3950 gauss) is $(461.75 \pm 0.02) \times 10^6$ sec.$^{-1}$ This precise agreement shows that the quantum-mechanical expressions for the variation of the magnetic levels in external magnetic fields must be accurate to at least one part in ten thousand. Thus one can use the Zeeman effect to calibrate a magnetic field for other purposes, such as the more exact measurement of nuclear moments.

One of the most important results from these studies is the value of the ratio of the hyperfine structure separations of the lithium isotopes (145). From this value can be calculated the ratio of the nuclear magnetic moments on the assumption that only electromagnetic interactions between the electrons and the nucleus have an effect on the atomic energy levels. The value obtained in this way is $\mu_7/\mu_6 = 3.9610 \pm 0.0004$. The directly determined value, from molecular-beam experiments, is 3.9601 ± 0.0015. This indicates that the assumption is valid. In the case of rubidium, the agreement is not quite so good: 2.0261 ± 0.0003 for the atomic-beam value (172),—Millman and Fox (168) obtained 2.026 by the method of zero moments,—and 2.238 ± 0.010 for the molecular-beam value. Millman and Kusch point out that because of the great experimental difficulties of the latter experiment, the discrepancy does not give conclusive evidence of a non-electromagnetic interaction between the electron and the nucleus.

During the past year, Millman and Kusch (173) have combined the atomic- and molecular-beam magnetic-resonance experiments to obtain more precise values of the nuclear magnetic moments. The accuracy of all the previous moment measurements was limited by the uncertainty of the strength of the homogeneous magnetic field in which the transitions took place. It was possible to obtain moment ratios much more accurately than the moments themselves, for these depend on a frequency ratio in a constant magnetic field. However, the studies of the radiofrequency spectra show that it is possible to make very precise measurements of the hyperfine structure separations from lines the positions of which are practically independent of the field. The frequencies of lines arising from certain other transitions show a very marked dependence upon the field strength and the hyperfine structure separation, a dependence the mathematical form of which is known. Thus it is possible to calculate the field strength from the frequency of the spectral line (see reference 185 for a justification of this calculation).

Millman and Kusch calibrated the field and measured the nuclear moments in the same experiment. They used an oven with two compartments connected only at the slit. In one was placed the substance (Na^{23}, Rb^{85}, or Cs^{133}) used for measuring the field; in the other, the compound containing the nucleus the moment of which was desired. This compound contained also, in every case, an alkali atom, so that the Taylor gauge could be used as the detector.

For the important case of hydrogen, the beam consisted of sodium hydroxide or potassium hydroxide. The magnetic field was kept at a constant value, and the frequency changed so as to get the minimum corresponding to the Larmor frequency of the proton moment. Before and after every experiment, the field was calibrated by running through the radiofrequency spectrum of one of the reference materials. The value obtained in this way for the proton moment was 2.7896 ± 0.0008 nuclear magnetons. This value was multiplied by the previously obtained ratio of the deuteron and proton moments to give the former a magnitude of 0.8565 ± 0.0004. The difference between these two is -1.933 ± 0.001 nuclear magnetons, a result to be compared with the neutron moment of -1.935 ± 0.02 obtained by Alvarez and Bloch (1).

TABLE 1

Nuclear moment values obtained by molecular-beam methods

NUCLEUS	i	$\Delta\nu$	μ	SIGN OF μ	METHOD*	DATE	REFERENCE
H^1	1/2		2.5 ± 10%		D	1933	(53, 58, 77)
			3.25 ± 10%		D	1934	(197)
		0.0482	2.85 ± 0.15	+	Z	1936	(121, 122, 199)
			2.46 ± 3%		D	1937	(55)
			2.785 ± 0.02	+	M	1939	(124, 125)
			2.7896 ± 0.0008		MA	1941	(173)
H^2			0.7		D	1933	(60, 61, 62)
	2/2				S	1933	(152)
	2/2				S	1934	(182)
			0.75 ± 0.2		D	1934	(198)
		0.0108	0.85 ± 0.03	+	Z	1936	(120, 122)
			0.855 ± 0.006	+	M	1939	(124, 125)
			0.8565 ± 0.0004		MA	1941	(173)
Li^6	≥2/2		0.5−0.8		Z	1935	(67)
			0.6±0.2	+	S	1936	(226)
	2/2	0.0077	0.85		Z	1936	(157, 158)
				+	Z	1938	(82)
			0.820 ± 0.005		M	1938	(200, 201)
				+	M	1939	(167)
		0.007613			A	1940	(145)
			0.8213 ± 0.0005		MA	1941	(173)
Li^7	3/2		3.29		S	1932	(84)
	3/2				S	1933	(86)
	3/2	0.0267	3.20		Z	1935	(67)
			3.33		T	1936	(7)
				+	Z	1937	(178)
			3.250 ± 0.0016		M	1938	(200, 201)
				+	M	1939	(167)
		0.026805			A	1940	(145)
			3.2532 ± 0.0015		MA	1941	(173)
Be^9	3/2?		1.176 ± 0.005	−	M, MA	1939	(142, 173)
B^{10}	2/2?		0.598 ± 0.003	+	M, MA	1939	(173, 175)
B^{11}	3/2?		2.686 ± 0.008	+	M, MA	1939	(173, 175)
C^{13}	1/2				T	1940	(99)
			0.701 ± 0.002	+	M	1940	(93, 95)
N^{14}	1		≤0.2		S	1933	(5)
			0.402 ± 0.002	+	M	1938	(143, 174)
			0.403 ± 0.002		MA	1941	(173)
N^{15}	1/2		0.280 ± 0.003	??	M, MA	1940	(173, 260)

TABLE 1—*Continued*

NUCLEUS	i	$\Delta\nu$	μ	SIGN OF μ	METHOD*	DATE	REFERENCE
F^{19}	1/2				S	1929	(79)
			2.622 (with Taylor gauge)		M	1938	(200, 201)
			2.623 (with Pirani gauge)		M	1939	(124, 125)
				+	M	1939	(167)
			2.625 ± 0.003		MA	1941	(173)
Na^{23}	3/2				D	1933	(195, 196)
	3/2				S	1933	(106)
	3/2		~2.6		S	1933	(248)
	3/2				S	1934	(43, 148)
	3/2	0.0596	2.08		Z	1935	(67)
				+	Z	1937	(245)
			2.216	+	M	1938	(143, 170)
		0.059103			A	1940	(172)
			2.215 ± 0.002		MA	1941	(173)
Al^{27}	9/2		3.6−4.1		S	1938	(104)
	5/2		3.7		S	1938	(97)
	5/2		3.630 ± 0.010	+	M, MA	1939	(171, 173)
Cl^{35}	5/2				S	1930	(48)
			1.368 ± 0.005	+	M, MA	1939	(140, 141, 173)
Cl^{37}	5/2				S	1940	(229)
			1.136 ± 0.005	+	M, MA	1939	(140, 141, 173, 230)
K^{39}	3/2	0.0152	0.38		Z	1934	(166, 169)
			0.10		T	1935	(81)
	≥3/2		0.43−0.47	−	S	1935	(102, 103)
	3/2	0.0154	0.397		Z	1935	(67)
				+	Z	1937	(245)
		0.0156	0.3−0.4	+	S	1937	(164)
			0.391 ± 0.002	+	M	1938	(143, 170)
		0.015403			A	1940	(145)
K^{40}	4		1.290	−	M	1941	(259)
K^{41}	>1/2				Z	1935	(166)
	≤5/2				Z	1935	(67)
	3/2	0.00853	0.22		Z	1936	(156)
				+	Z	1938	(82)
		0.008474			A	1940	(145)
			0.215 ± 0.002		MA	1941	(173)
Ga^{69}	3/2				S	1932	(27, 100, 225)
	3/2		2.11		Z	1940	(207)

TABLE 1—*Continued*

NUCLEUS	i	$\Delta\nu$	μ	SIGN OF μ	METHOD*	DATE	REFERENCE
Ga^{71}	3/2				S	1932	(27, 100, 225)
	3/2		2.69		Z	1940	(207)
Rb^{85}	3/2				S	1933	(101)
	5/2		1.4		S	1933	(137)
	5/2	0.1018	1.44 ± 1%		Z	1936	(168)
				+	Z	1937	(178)
			1.8 ± 30%		Modified Z	1937	(176)
			1.340 ± 0.005	+	M, MA	1939	(140, 141, 173)
		0.10127			A	1940	(172)
Rb^{87}	5/2				S	1933	(101)
	3/2		2.8		S	1933	(137)
		0.229	2.92 ± 1%		Z	1936	(168)
				+	Z	1937	(178)
			2.733 ± 0.009	+	M, MA	1939	(140, 141, 173)
		0.22797			A	1940	(172)
In^{113}		No trace found			Modified Z	1938	(177)
	9/2		0.998 × μ_{115}	+	A	1941	(92)
In^{115}			5.3 ± 0.5		S	1937	(227)
	9/2	0.381	6.40 ± 0.20		Modified Z	1938	(177)
			5.43 ± 0.03	+	A	1941	(91)
Cs^{133}	7/2	0.295			Z	1934	(28)
		0.3067	2.38–3.01		S	1935	(85)
	7/2				S	1936	(138)
	7/2	0.307	2.72 ± 1%		Z	1936	(168)
				+	Z	1937	(178)
	7/2				S	1937	(66)
			2.558 ± 0.007	+	M, MA	1939	(139, 143, 173)
		0.30661			A	1940	(172)
Ba^{135}	3/2		0.837 ± 0.003	+	M	1940	(94, 95)
Ba^{137}	3/2		0.936 ± 0.003	+	M	1940	(94, 95)
(H^1)$_2$	$\mu_R = 0.36-0.93$				T	1933	(253)
	$\mu_R = 0.8-0.9$				D	1933	(53, 58)
	$\mu_R = 0.8787 \pm 0.0070\ (j = 1)$ $\mu_R = 1.757 \pm 0.014\ (j = 2)$				M	1939	(204)
	$\mu_R = 0.885$				T	1941	(25)
(H^{1}H^2)	$\mu_R = 0.6601 \pm 0.0050$				M	1939	(204)
(H^2)$_2$	$\mu_R = 0.4406 \pm 0.0030$				M	1939	(204)
H^2	$Q = (2.73 \times 10^{-27}) \pm 2\%$ cm.^{2}				M	1939	(123, 126)

TABLE 1—*Concluded*

NUCLEUS		METHOD*	DATE	REFERENCE
Ga^{69}	$Q = 1 \times 10^{-24}$	S	1936	(225)
	0.20×10^{-24}	Z	1940	(207)
Ga^{71}	$Q = 0.5 \times 10^{-24}$	S	1936	(225)
	0.13×10^{-24}	Z	1940	(207)
In^{115}	$Q = 1.0 \times 10^{-24}$	S	1936	(6)
	$(0.8 \pm 0.2) \times 10^{-24}$	S	1937	(227)
	0.84×10^{-24}	Z	1939	(89)

* A = atomic magnetic resonance.
D = deflection.
M = molecular magnetic resonance.
MA = molecular and atomic magnetic resonance combined.
S = spectroscopy.
T = theory.
Z = zero moments.

The proton moment has now been measured more precisely than any other; therefore all other moments have been referred to it. Li^7 is used as a secondary standard because of the ease of making a beam of these atoms and of detecting it. This beautiful and epoch-making work of Rabi and his collaborators has brought the accuracy of the value of the proton moment up to that of many of the other physical constants. The discrepancy between this value and the lower one obtained by Stern and his colleagues may have physical significance, although at present it cannot be explained in a completely satisfactory manner (233a, 264).

In table 1 is given a list of all the nuclear moment values obtained by molecular-beam methods. These have not been corrected for the diamagnetism of the atoms. The spectroscopically obtained values for the same nuclei are given for comparison.

VIII. ELECTRIC DEFLECTION

In 1921, Kallmann and Reich (117) suggested that the electrical properties of molecules could be studied by a molecular-beam method. Stern (237) also pointed out that the same method was applicable to both the electric and magnetic cases. The two experiments are similar in principle, but in actual practice the former is far more complicated and therefore less amenable to interpretation.

In the first place, atoms are so symmetrical electrically that they have no electric moment (see later, however). Thus the investigations are almost entirely limited to beams of molecules. These molecules will rotate with the axis of rotation in any direction whatsoever. We must distinguish, therefore, two cases: that in which the electric moment of the molecule is perpendicular to the axis of rotation (dumbbell molecules; example, hydrogen chloride) and that in which the moment is not perpendicular to the axis of rotation. In the first case, in the absence of a field, the moment will average to zero because of the rotation. However, in an electric field the rotation will no longer be uniform, and therefore there will be a time-averaged moment proportional to the electric field strength. Its value will be from 1/1000 to 1/100 of the permanent moment. Since the energy of the molecule in the field, and also its deflection in an inhomogeneous electric field, are proportional both to the field strength and to the average moment, the deflection and energy will be proportional to the square of the field strength. This is the quadratic Stark effect.

In order to predict the shape of the deflection pattern, one must average the individual deflections over all possible velocities of the molecules, all possible rotational states, and all possible directions of the axis of rotation. A slight consideration will show that the molecular beam will not be split, but will simply be broadened. This broadening is not quite symmetric; furthermore, with an infinitely narrow beam and detector, it would be found that the position of maximum intensity had been shifted by a very small amount. However, as ordinarily used, the molecular-ray apparatus has insufficient resolution to detect this shift. Nevertheless, from the weakening of the beam as it passes through the electric field, it is possible to calculate the electric moment (see 218, 220, and Fraser's book).

In the above considerations there has been omitted one thing which must be taken into account. The electron cloud surrounding the nuclei in the molecule will be distorted somewhat by the electric field; therefore there will be an induced moment proportional to the field strength. The effect of this will be to displace the whole deflection pattern toward the direction of higher inhomogeneity.

Experiments have been performed on hydrogen chloride (51, 163, 208), the alkali halides (70, 210, 217, 256), and various organic molecules (49, 50, 63, 254) for the purpose of measuring their electric moments. The apparatus was in every case essentially a Stern–Gerlach apparatus. The fields were produced either by parallel-plate condensers (188) or by cylindrical condensers with the beam passing very close to and parallel to the inner electrode. Quantitative measurements were attempted in only a few cases. While the results of various investigators do not agree very well, still the disagreement is less than the experimental error. The values agree roughly with those obtained by other methods.

If the molecular moment has a component parallel to the axis of rotation, the moment will not average to zero. Thus there will be a permanent moment, and the energy and deflection will be proportional to the first power of the field. This linear effect, being of the first order, is, of course, much greater than the quadratic effect. The theory has been quite completely worked out by Scheffers (220). In practice it can be applied only to symmetric molecules, because the shape of the deflection pattern must be known beforehand. That is, the direction of the moment with respect to the axis of rotation must be known; otherwise the interpretation of the deflection pattern is practically impossible.

The only quantitative molecular-beam experiment on a molecule exhibiting the linear Stark effect was performed by Scheffers (221) on ammonia. He found, as he had predicted, that the deflection was linear with the field. However, the value of the moment of the ammonia molecule was only one-third that obtained by other methods. Scheffers insists that this discrepancy is definitely not due to experimental error.

As has been mentioned, atoms have no permanent moments. However, with atoms, just as with molecules, there is the possibility of induced moments. Scheffers and Stark (219, 222, 223) deflected beams of lithium, potassium, cesium, hydrogen, and oxygen atoms in an inhomogeneous electric field. In every case the beam was shifted bodily in the direction of the gradient. Thus, from the simple measurement of the displacement, it is possible to calculate the polarizability $\alpha = \mu/E$. The values so obtained were of the same order of magnitude as the theoretical values, but the agreement was not particularly good.

IX. MISCELLANEOUS MOLECULAR-BEAM EXPERIMENTS

A. Life-time of excited states

Dunoyer's (38) main application of his method was to the study of spectroscopy. He found that if a beam of sodium atoms was illuminated with sodium light, it luminesced because of the resonance radiation. The lateral boundaries of the irradiated portion of the beam were very sharp, and those in the direction of motion were equally distinct. This gave direct proof that the time of excitation was very small, a result in agreement with the Drude theory.

Koenig and Ellett (136) have actually measured this time for cadmium resonance radiation; usually it is of the order of 10^{-8} sec., but in this case spectroscopic results led to the belief that it was much longer. They irradiated a part of a beam of cadmium perpendicularly with a very narrow ray of the line λ 3261. This portion was screened from a camera, yet film blackening was found for a distance of 4 mm. beyond the illuminated part. The temperature, and therefore the average velocity of the beam, were known. From this it was determined that the life-time was of the order of 10^{-6} sec.

B. Spectral lines

The molecular-beam method has a very great advantage in the field of spectroscopy. Because there are practically no collisons in the beam and very little mutual interaction, and because the velocities of the beam particles are essentially parallel, there will be practically no pressure broadening of the spectral lines emitted by an excited molecular beam. If the light is viewed at right angles to the direction of motion, there will also be almost no Doppler broadening; this alone is equivalent to having a spectral source at a temperature of only a few degrees Absolute. Thus such a light source is adapted for studies of the hyperfine structure of spectral lines.

Several methods of exciting the radiation have been used. Bogros (17), Dobrezov and Terenin (35), and Jackson and Kuhn (102, 104) have used optical excitation, and have studied either the resonance radiation itself (emission spectrum) or the absorption due to the passage of the original light through the beam. The intensity of the spectrum produced in this way is not very great. Bogros and Esclangon (18) increased the intensity very much by stimulating emission with a high-frequency discharge. For this purpose it was necessary that the beam pass through a low pressure (2×10^{-3} mm. of mercury) of argon or nitrogen which conducts the discharge. They found the lines to be just as sharp as the absorption lines produced in a beam in a good vacuum. Minkowski and Bruck (179) have obtained very high resolution by bombarding a sharp beam of cadmium with electrons. Meissner (164) has made some very careful spectral measurements on beams of potassium, using optical excitation. A value of the nuclear magnetic moment of K^{39} was obtained from the hyperfine structure which agreed very well with that obtained by magnetic deflection.

C. Ionization

Closely allied to the spectroscopic problem is that of the ionization of a beam of neutral particles. It is often of importance to obtain a large positive-ion current in a good vacuum. This may be done easily with the use of a molecular beam. Planiol (187) and Sasaki and Nishibori (216) have developed the method in considerable detail. Planiol obtained currents as high as 40 milliamperes from the collision of mutually perpendicular rays of cadmium atoms and electrons. Bleakney, Blewett, Sherr, and Smoluchowski (15) and Ditchburn and Arnot (34) have applied this same technique to obtain ions for a mass spectrograph, following the earlier example of Smyth (233).

Jewitt (105) and Fraser and Jewitt (71) have used a molecular-beam method to measure the ionization potential of formaldehyde, the halogen-substituted methanes, and the free radicals methyl and ethyl. The beams were passed either into a Kingdon gauge or an ionization gauge. Curves were plotted of beam intensity against anode voltage and extrapolated to zero to get the ionization potential. The results for the free radicals were in agreement with estimates made from other methods.

D. Degree of dissociation

As has already been described, Zartman and Ko made an analysis of the velocity distribution in a beam of bismuth. They found that in order to explain their results, they must assume the presence of diatomic bismuth molecules, and perhaps even Bi_8.

Lewis (153) has perfected a much more sensitive method of determining the degree of dissociation of non-magnetic molecules composed of atoms with a magnetic moment. The experiment is very simple. A beam is passed through a Stern–Gerlach field and its intensity measured at the undeflected position. Then the field is decreased to zero and the intensity determined again. The latter measurement is proportional to the total number of particles in the beam; the former only to the number of molecules, since all the atoms were deflected in the field. If the same process is repeated for a whole range of temperatures, it is possible to obtain the degree of dissociation as a function of temperature, and from this to calculate the equilibrium constant and the heat of dissociation.

Lewis studied the equilibrium of Li_2, Na_2, and K_2 in this way. In a similar manner Leu (151) determined the heat of dissociation of Bi_2.

E. Chemical studies

Molecular beams have been used by Sasaki (216) and his coworkers in a series of chemical studies upon the mechanism of reactions and the direct determination of radii of spheres of action between combining atoms. These unique experiments are mentioned because they indicate the power of the method and will perhaps suggest other applications. For instance, it might be possible to study the rates of some first-order chemical reactions by measuring the decrease in the number of particles in the beam as a function of the distance from the source. The difficulty due to chain reactions experienced in the ordinary method would not appear in the beam method.

F. Einstein recoil

Frisch (74) illuminated a very narrow ribbon-shaped beam of sodium sidewise with sodium light, and found that the beam was displaced by about the correct amount and in the correct direction; when the beam was illuminated edgewise, it was broadened a little. This is presumptive evidence of the existence of the Einstein recoil. This experiment, although difficult to perform, is very simple in principle, and provides another example of the power of the molecular-ray method to give results of a fundamental nature.

G. High-energy beams

With all the experiments described above, the beams were produced by the temperature motions of the gaseous materials, and therefore had velocities appropriate to the temperature of the oven. Recently an entirely new method of production of molecular beams has been developed which gives particles of very high energy (10, 115, 116, 118, 214, 215). Positive ions are to a large extent neutralized when passing through low pressures of the un-ionized gas. It is found that very many of them change neither the direction nor the magnitude of their velocities during this discharging process. Thus, if a focused beam of positive ions is neutralized and then collimated again, and if all the remaining ions are deflected to the side, there will remain a beam of neutral particles of essentially homogeneous but controllable velocity. The beams produced in this way have energies of the order of a few hundred electron volts; in contrast to this, particles at room temperature have energies 1/40 of an electron volt.

The detection of these beams is not a difficult matter; two methods have been used quite successfully. Beeck (12) and others (2, 36, 160, 215) have used a thermopile which theoretically could give the absolute intensity. The other method, which at present is limited to qualitative results, is simply the measurement of the number of secondary electrons emitted from a negative plate by the impact of the molecules (22, 115, 184, 214).

Amdur and Pearlman (3) have measured the collision cross sections of such beams of helium in helium and of hydrogen atoms in molecular hydrogen. The beam energies varied from 200 to 1000 electron volts. With such energies, a study of the scattering reveals information about the repulsive forces between atoms. They found a marked lowering of the cross sections with increasing energies. This is not in agreement with the hard-sphere theory or the theory of Lennard-Jones. For the case of helium, they calculated a potential for the repulsive force which leads to the observed results.

Studies have been made by Beeck (8, 9, 12) and Varney (250) on the ionization produced in various gases by fast beams. The latter found that the energies of the beams must be about three times the ionization potential before ions are produced.

These fast beams can also be used to produce optical excitation. Maurer (160) has stimulated emission of thirteen lines of the helium spectrum by passing beams of helium atoms through helium. The light intensity was strictly proportional to the beam intensity, and, except for the singlet principal series, proportional to the pressure. The excitation in the range from a few hundred to 6000 volts was very similar to that produced by electrons in the range 0–40 volts. Gailer (78) studied the excitation of magnesium by helium and hydrogen atoms. He found arc and spark lines of magnesium, but no lines of helium or hydrogen. This is in agreement with the Döpel impact model.

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REFERENCES

(1) Alvarez, L. W.: Phys. Rev. **57**, 352 (1940).
Alvarez, L. W., and Bloch, F.: Phys. Rev. **57**, 111 (1940).
(2) Amdur, I., and Pearlman, H.: Rev. Sci. Instruments **10**, 174 (1939).
(3) Amdur, I., and Pearlman, H.: J. Chem. Phys. **8**, 7 (1940); **9**, 503 (1941).
(4) Anthony, W. A.: Trans. Am. Inst. Elec. Engrs. **11**, 142 (1894).
(5) Bacher, R. F.: Phys. Rev. **43**, 1001 (1933).
(6) Bacher, R. F., and Tomboulian, D. K.: Phys. Rev. **50**, 1096 (1936).
(7) Bartlett, J. H., Jr., Gibbons, J. J., Jr., and Watson, R. E.: Phys. Rev. **50**, 315 (1936).
(8) Beeck, Otto: Z. Physik **76**, 799 (1932).
(9) Beeck, Otto: Proc. Natl. Acad. Sci. U. S. **18**, 311 (1932).
(10) Beeck, Otto: Ann. Physik **19**, 121 (1934).
(11) Beeck, Otto: J. Chem. Phys. **4**, 680 (1936); **5**, 268 (1937).
(12) Beeck, O., and Wayland, H.: Ann. Physik **19**, 129 (1934).
(13) Bessey, William H.: Dissertation, Carnegie Institute of Technology, 1940; Phys. Rev. **59**, 459 (1941).
(14) Bielz, Fritz: Z. Physik **32**, 81 (1925).
(15) Bleakney, W., Blewett, J. P., Sherr, R., and Smoluchowski, R.: Phys. Rev. **50**, 545 (1936).
(16) Bloch, F., and Seigert, A.: Phys. Rev. **57**, 522 (1940).
(17) Bogros, A.: Compt. rend. **183**, 124 (1926); **190**, 1185 (1930); Ann. phys. **17**, 199 (1932).
(18) Bogros, A., and Esclangon, Felix: Compt. rend. **195**, 368 (1932).
(19) Born, Max: Physik. Z. **21**, 578 (1920).
(20) Brady, James J.: Phys. Rev. **41**, 613 (1932); **47**, 197 (1935).

(21) Brandt, E.: Z. Physik **92**, 640 (1934).
(22) Brasefield, C. J.: Phys. Rev. **44**, 1002 (1934).
(23) Breit, G., and Rabi, I. I.: Phys. Rev. **38**, 2082 (1931).
(24) Broadway, L. F.: Proc. Roy. Soc. (London) **141**, 634 (1933); Z. Physik **93**, 395 (1935).
(25) Brooks, Harvey: Phys. Rev. **59**, 925 (1941); **60**, 168 (1941).
(26) Caldirola, P.: Nuovo cimento **16**, 242 (1939).
(27) Campbell, J. S.: Nature **131**, 204 (1933).
(28) Cohen, V. W.: Phys. Rev. **46**, 713 (1934).
(29) Cohen, V. W., and Ellett, A.: Phys. Rev. **52**, 502 (1937).
(30) Copley, M. J., and Deitz, V.: Rev. Sci. Instruments **8**, 314 (1937).
(31) Copley, M. J., and Phipps, T. E.: Phys. Rev. **45**, 344 (1934); **46**, 144 (1934); **48**, 960 (1935).
(32) Costa, J. L., Smyth, H. D., and Compton, K. T.: Phys. Rev. **30**, 349 (1927).
(33) Devonshire, A. F.: Proc. Roy. Soc. (London) **A156**, 37 (1936).
(34) Ditchburn, R. W., and Arnot, F. L.: Proc. Roy. Soc. (London) **A123**, 516 (1929).
(35) Dobrezov, L., and Terenin, A.: Naturwissenschaften **16**, 656 (1928).
(36) Döpel, R.: Ann. Physik **16**, 1 (1933).
(37) Dunoyer, L.: Compt. rend. **152**, 592 (1911); Le radium **8**, 142 (1911).
(38) Dunoyer, L.: Compt. rend. **157**, 1068 (1913); Le radium **10**, 400 (1913).
(39) Eldridge, J. A.: Phys. Rev. **30**, 931 (1927).
(40) Eldridge, J. A.: Phys. Rev. **40**, 1050 (1932); **49**, 411 (1936).
(41) Ellett, A., and Cohen, V.: Phys. Rev. **51**, 64 (1937).
(42) Ellett, A., and Cohen, V.: Phys. Rev. **52**, 509 (1937).
(43) Ellett, A., and Heydenberg, N. P.: Phys. Rev. **46**, 583 (1934).
(44) Ellett, A., and Olson, H. F.: Phys. Rev. **31**, 643 (1928).
(45) Ellett, A., and Olson, H. F.: Phys. Rev. **33**, 118 (1929).
(46) Ellett, A., and Zahl, H. A.: Phys. Rev. **33**, 124 (1929).
(47) Ellett, A., Zahl, H. A., and Olson, H. F.: Phys. Rev. **34**, 492 (1929).
(48) Elliott, A.: Proc. Roy. Soc. (London) **A127**, 638 (1930).
(49) Estermann, I.: Z. physik. Chem. **B1**, 161 (1928).
(50) Estermann, I.: Z. physik. Chem. **B2**, 287 (1929).
(51) Estermann, I., and Fraser, R. G. J.: J. Chem. Phys. **1**, 390 (1933).
(52) Estermann, I., Frisch, R., and Stern, O.: Z. Physik **73**, 348 (1931).
(53) Estermann, I., Frisch, R., and Stern, O.: Nature **132**, 169 (1933).
(54) Estermann, I., Simpson, O. C., and Stern, O.: Phys. Rev. **51**, 64 (1937).
(55) Estermann, I., Simpson, O. C., and Stern, O.: Phys. Rev. **52**, 535 (1937).
(56) Estermann, I., Simpson, O. C., and Stern, O.: Phys. Rev. **53**, 947 (1938).
(57) Estermann, I., and Stern, O.: Z. Physik **61**, 95 (1930).
(58) Estermann, I., and Stern, O.: Z. Physik **85**, 17 (1933).
(59) Estermann, I., and Stern, O.: Z. Physik **85**, 135 (1933).
(60) Estermann, I., and Stern, O.: Z. Physik **86**, 132 (1933).
(61) Estermann, I., and Stern, O.: Nature **133**, 911 (1934).
(62) Estermann, I., and Stern, O.: Phys. Rev. **45**, 761 (1934).
(63) Estermann, I., and Wohlwill, M.: Z. physik. Chem. **B20**, 195 (1933).
(64) Ewald, P. P., and Renninger, M.: *International Conference on Physics, London,* 1934. Vol. II, *Solid State of Matter*, p. 62. London (1935).
(65) Fermi, E.: Z. Physik. **60**, 320 (1930).
Fermi, E., and Segré, E.: Z. Physik. **82**, 729 (1933).
(66) Fölsche, Trutz: Z. Physik. **105**, 133 (1937).
(67) Fox, Marvin, and Rabi, I. I.: Phys. Rev. **48**, 746 (1935).
(68) Fraser, R. G. J.: Trans. Faraday Soc. **30**, 182 (1934).
(69) Fraser, R. G. J., and Broadway, L. F.: Proc. Roy. Soc. (London) **A141**, 626 (1933).
(70) Fraser, R. G. J., and Hughes, J. V.: J. Chem. Phys. **4**, 730 (1936).
(71) Fraser, R. G. J., and Jewitt, T. N.: Phys. Rev. **50**, 1091 (1936).

(72) Fraser, R. G. J., Massey, H. S. W., and Mohr, C. B. O.: Z. Physik **97**, 740 (1935).
(73) Frisch, R.: Z. Physik. **84**, 443 (1933).
(74) Frisch, R.: Z. Physik. **86**, 42 (1933).
(75) Frisch, R., and Segré, E.: Z. Physik **80**, 610 (1933).
(76) Frisch, R., and Stern, O.: Z. Physik **84**, 430 (1933).
(77) Frisch, R., and Stern, O.: Z. Physik **85**, 4 (1933).
(78) Gailer, K.: Z. Physik **103**, 303 (1936).
(79) Gale, H. G., and Monk, G. S.: Astrophys. J. **69**, 77 (1929).
(80) Gerlach, W., and Stern, O.: Z. Physik **8**, 110 (1922); **9**, 349, 353 (1922); Ann. Physik **74**, 673 (1924).
(81) Gibbons, J. J., and Bartlett, J. H.: Phys. Rev. **47**, 340 (1935).
(82) Gorham, J. E.: Phys. Rev. **53**, 563 (1938).
(83) Goudsmit, S.: Phys. Rev. **43**, 636 (1933).
(84) Granath, L. P.: Phys. Rev. **42**, 44 (1932).
(85) Granath, L. P., and Stranathan, R. K.: Phys. Rev. **48**, 725 (1935).
(86) Gray, N. M.: Phys. Rev. **43**, 1044 (1933); **44**, 570 (1933).
(87) Guthrie, A. N.: Phys. Rev. **49**, 868 (1936).
(88) Güttinger, P.: Z. Physik **73**, 169 (1931).
(89) Hamilton, D. R.: Phys. Rev. **56**, 30 (1939).
(90) Hancox, Robert Rex: Phys. Rev. **42**, 864 (1932).
(91) Hardy, T. C.: Phys. Rev. **59**, 686 (1941).
(92) Hardy, T. C., and Millman, S.: Phys. Rev. **60**, 167 (1941).
(93) Hay, Robert H.: Phys. Rev. **58**, 180 (1940).
(94) Hay, Robert H.: Phys. Rev. **59**, 686 (1941).
(95) Hay, Robert H.: Phys. Rev. **60**, 75 (1941).
(96) Hendricks, J. O., Phipps, T. E., and Copley, M. J.: J. Chem. Phys. **5**, 868 (1937).
(97) Heyden, M., and Ritschl, R.: Z. Physik **108**, 739 (1938).
(98) Holst, G., and Clausing, P.: Physica **6**, 48 (1926).
(99) Inglis, D. R.: Phys. Rev. **58**, 577 (1940).
(100) Jackson, D. A.: Z. Physik **75**, 229 (1932).
(101) Jackson, D. A.: Proc. Roy. Soc. (London) **A139**, 673 (1933).
(102) Jackson, D. A., and Kuhn, H.: Proc. Roy. Soc. (London) **A148**, 335 (1935).
(103) Jackson, D. A., and Kuhn, H.: Nature **137**, 108 (1936).
(104) Jackson, D. A., and Kuhn, H.: Proc. Roy. Soc. (London) **A164**, 48 (1938).
(105) Jewitt, T. N.: Phys. Rev. **46**, 616 (1934).
(106) Joffe, J.: Phys. Rev. **45**, 468 (1934).
Joffe, J., and Urey, H. C.: Phys. Rev. **43**, 761 (1933).
(107) Johnson, M. C., and Starkey, T. V.: Proc. Roy. Soc. (London) **A140**, 126 (1933).
(108) Johnson, T. H.: Phys. Rev. **27**, 519 (1926); Nature **119**, 745 (1927); Phys. Rev. **31**, 103 (1928).
(109) Johnson, T. H.: J. Franklin Inst. **206**, 301 (1928); Phys. Rev. **31**, 1122 (1928); J. Franklin Inst. **207**, 629, 639 (1929).
(110) Johnson, T. H.: Phys. Rev. **35**, 650 (1930); J. Franklin Inst. **210**, 135 (1930); Phys. Rev. **35**, 1299, 1432 (1930).
(111) Johnson, T. H.: Phys. Rev. **37**, 847 (1931).
(112) Johnson, T. H.: Phys. Rev. **37**, 87 (1931); J. Franklin Inst. **212**, 507 (1931).
(113) Johnson, T. H.: Phys. Rev. **38**, 586 (1931).
(114) Josephy, B.: Z. Physik **80**, 755 (1933).
(115) Kallmann, H.: Z. Physik **61**, 61 (1930); **64**, 806 (1930).
(116) Kallmann, H., Lasareff, W., and Rosen, B.: Z. Physik **76**, 213 (1932).
(117) Kallmann, H., and Reiche, R.: Z. Physik **6**, 352 (1921).
(118) Kallmann, H., and Rosen, B.: Naturwissenschaften **18**, 867 (1930).
(119) Kellogg, J. M. B.: Phys. Rev. **41**, 635 (1932).
(120) Kellogg, J. M. B., Rabi, I. I., and Zacharias, J. R.: Phys. Rev. **49**, 867 (1936).

(121) KELLOGG, J. M. B., RABI, I. I., AND ZACHARIAS, J. R.: Phys. Rev. **50**, 396 (1936).
(122) KELLOGG, J. M. B., RABI, I. I., AND ZACHARIAS, J. R.: Phys. Rev. **50**, 472 (1936).
(123) KELLOGG, J. M. B., RABI, I. I., RAMSEY, N. F., JR., AND ZACHARIAS, J. R.: Phys. Rev. **55**, 318 (1939).
(124) KELLOGG, J. M. B., RABI, I. I., RAMSEY, N. F., JR., AND ZACHARIAS, J. R.: Phys. Rev. **55**, 595 (1939).
(125) KELLOGG, J. M. B., RABI, I. I., RAMSEY, N. F., JR., AND ZACHARIAS, J. R.: Phys. Rev. **56**, 728 (1939).
(126) KELLOGG, J. M. B., RABI, I. I., RAMSEY, N. F., JR., AND ZACHARIAS, J. R.: Phys. Rev. **57**, 677 (1940).
(127) KELLOGG, J. M. B., AND RAMSEY, N. F.: Phys. Rev. **53**, 331 (1938).
(128) KERSCHBAUM, HANS: Ann. Physik **2**, 201 (1929).
(129) KERSCHBAUM, HANS: Ann. Physik **2**, 213 (1929).
(130) KNAUER, F.: Z. Physik **80**, 80 (1933); **90**, 559 (1934); **93**, 397 (1935).
(131) KNAUER, F., AND STERN, O.: Z. Physik **39**, 764 (1926).
(132) KNAUER, F., AND STERN, O.: Z. Physik **53**, 766 (1929).
(133) KNAUER, F., AND STERN, O.: Z. Physik **53**, 779 (1929).
(134) KO, CHENG CHUAN: Phys. Rev. **44**, 129 (1933); J. Franklin Inst. **217**, 173 (1934).
(135) KODERA, K.: Bull. Chem. Soc. Japan **14**, 114, 141 (1939).
(136) KOENIG, H. D., AND ELLETT, A.: Phys. Rev. **37**, 1685 (1931).
(137) KOPFERMANN, HANS: Z. Physik **83**, 417 (1933).
(138) KOPFERMANN, HANS, AND KRÜGER, H.: Z. Physik **102**, 527 (1936).
(139) KUSCH, P., AND MILLMAN, S.: Phys. Rev. **55**, 596 (1939).
(140) KUSCH, P., AND MILLMAN, S.: Phys. Rev. **55**, 680 (1939).
(141) KUSCH, P., AND MILLMAN, S.: Phys. Rev. **56**, 527 (1939).
(142) KUSCH, P., MILLMAN, S., AND RABI, I. I.: Phys. Rev. **55**, 666 (1939).
(143) KUSCH, P., MILLMAN, S., AND RABI, I. I.: Phys. Rev. **55**, 1176 (1939).
(144) KUSCH, P., MILLMAN, S., AND RABI, I. I.: Phys. Rev. **57**, 352 (1940).
(145) KUSCH, P., MILLMAN, S., AND RABI, I. I.: Phys. Rev. **57**, 765 (1940).
(146) LAMMERT, BERTHOLD: Z. Physik **56**, 244 (1929).
(147) LANGMUIR, I., AND VILLARS, D. S.: J. Am. Chem. Soc. **53**, 486 (1931).
(148) LARRICK, LEWIS: Phys. Rev. **46**, 581 (1934).
(149) LENNARD-JONES, J. E., AND DEVONSHIRE, A. F.: Nature **137**, 1069 (1936); Proc. Roy. Soc. (London) **A158**, 253 (1937).
(150) LENZ, W.: Z. Physik **92**, 631 (1934).
(151) LEU, ALFRED: Z. Physik **41**, 551 (1927); **49**, 498 (1928).
(152) LEWIS, G. N., AND ASHLEY, MURIEL: Phys. Rev. **43**, 837 (1933).
(153) LEWIS, L. C.: Z. Physik **69**, 786 (1931).
(154) MAIS, W. H.: Phys. Rev. **45**, 773 (1934).
(155) MAJORANA, ETTORE: Nuovo cimento **9**, 43 (1932).
(156) MANLEY, J. H.: Phys. Rev. **49**, 921 (1936).
(157) MANLEY, J. H., AND MILLMAN, S.: Phys. Rev. **50**, 380 (1936).
(158) MANLEY, J. H., AND MILLMAN, S.: Phys. Rev. **51**, 19 (1937).
(159) MASSEY, H. S. W., AND MOHR, C. B. O.: Proc. Roy. Soc. (London) **A141**, 434 (1933).
(160) MAURER, W.: Z. Physik **96**, 489 (1935).
(161) MAYER, HERBERT: Z. Physik **52**, 235 (1928).
(162) MAYER, HERBERT: Physik. Z. **36**, 463, 845 (1935); Z. techn. Physik **16**, 451 (1935).
(163) MCMILLAN, EDWIN: Phys. Rev. **42**, 905 (1932).
(164) MEISSNER, K. W., AND LUFT, K. F.: Z. Physik **106**, 362 (1937).
(165) MEISSNER, W., AND SCHEFFERS, H.: Physik. Z. **34**, 48, 245 (1933).
(166) MILLMAN, S.: Phys. Rev. **47**, 739 (1935).
(167) MILLMAN, S.: Phys. Rev. **55**, 628 (1939).
(168) MILLMAN, S., AND FOX, M.: Phys. Rev. **50**, 220 (1936).

(169) Millman, S., Fox, M., and Rabi, I. I.: Phys. Rev. **46,** 320 (1934).
(170) Millman, S., and Kusch, P.: Phys. Rev. **55,** 596 (1939).
(171) Millman, S., and Kusch, P.: Phys. Rev. **56,** 303 (1939).
(172) Millman, S., and Kusch, P.: Phys. Rev. **58,** 438 (1940).
(173) Millman, S., and Kusch, P.: Phys. Rev. **60,** 91 (1941).
(174) Millman, S., Kusch, P., and Rabi, I. I.: Phys. Rev. **54,** 968 (1938).
(175) Millman, S., Kusch, P., and Rabi, I. I.: Phys. Rev. **56,** 165 (1939).
(176) Millman, S., Rabi, I. I., and Zacharias, J. R.: Phys. Rev. **51,** 379 (1937).
(177) Millman, S., Rabi, I. I., and Zacharias, J. R.: Phys. Rev. **53,** 384 (1938).
(178) Millman, S., and Zacharias, J. R.: Phys. Rev. **51,** 1049 (1937).
(179) Minkowski, R., and Bruck, H.: Z. Physik **95,** 274, 284 (1935).
(180) Mizushima, San-ichiro: Physik. Z. **32,** 798 (1931).
(181) Motz, Lloyd, and Rose, M. E.: Phys. Rev. **50,** 348 (1936).
(182) Murphy, G. M., and Johnston, Helen: Phys. Rev. **46,** 95 (1934).
(183) Nordsieck, A.: Phys. Rev. **58,** 310 (1940).
(184) Oliphant, M. L. E.: Proc. Roy. Soc. (London) **A124,** 228 (1929).
(185) Phillips, M.: Phys. Rev. **60,** 100 (1941).
(186) Phipps, T. E., and Stern, O.: Z. Physik **73,** 185 (1931).
(187) Planiol, R.: Compt. rend. **200,** 539, 730 (1935); **202,** 1032 (1936); **204,** 1632 (1937); Ann. phys. **9,** 177 (1938).
(188) Rabi, I. I.: Nature **123,** 164 (1929); Z. Physik **54,** 190 (1929).
(189) Rabi, I. I.: Rev. Sci. Instruments **6,** 251 (1935).
(190) Rabi, I. I.: Phys. Rev. **47,** 334 (1935).
(191) Rabi, I. I.: Phys. Rev. **47,** 338 (1935).
(192) Rabi, I. I.: Phys. Rev. **49,** 324 (1936).
(193) Rabi, I. I.: Phys. Rev. **51,** 652 (1937).
(194) Rabi, I. I., and Cohen, V. W.: Phys. Rev. **43,** 377, 1044 (1933).
(195) Rabi, I. I., and Cohen, V. W.: Phys. Rev. **43,** 582 (1933).
(196) Rabi, I. I., and Cohen, V. W.: Phys. Rev. **46,** 707 (1934).
(197) Rabi, I. I., Kellogg, J. M. B., and Zacharias, J. R.: Phys. Rev. **46,** 157 (1934).
(198) Rabi, I. I., Kellogg, J. M. B., and Zacharias, J. R.: Phys. Rev. **46,** 163 (1934).
(199) Rabi, I. I., Kellogg, J. M. B., and Zacharias, J. R.: Phys. Rev. **49,** 421 (1936).
(200) Rabi, I. I., Millman, S., Kusch, P., and Zacharias, J. R.: Phys. Rev. **53,** 495 (1938).
(201) Rabi, I. I., Millman, S., Kusch, P., and Zacharias, J. R.: Phys. Rev. **55,** 526 (1939).
(202) Rabi, I. I., Zacharias, J. R., and Kellogg, J. M. B.: Phys. Rev. **49,** 200 (1936).
(203) Rabi, I. I., Zacharias, J. R., Millman, S., and Kusch, P.: Phys. Rev. **53,** 318 (1938).
(204) Ramsey, N. F., Jr.: Phys. Rev. **55,** 595 (1939); **58,** 226 (1940).
(205) Reimann, A. L.: Phys. Rev. **45,** 898 (1934).
(206) Renninger, M.: Z. Krist. **89,** 344 (1934).
(207) Renzetti, N. A.: Phys. Rev. **57,** 753 (1940).
(208) Rodebush, W. H., and Copley, M. J.: Phys. Rev. **33,** 1083 (1929).
(209) Rodebush, W. H., and Henry, W. F.: Phys. Rev. **39,** 386 (1932).
(210) Rodebush, W. H., Murray, L. A., and Bixler, M. E.: J. Chem. Phys. **4,** 372 (1936).
(211) Rosen, N., and Zener, C.: Phys. Rev. **40,** 502 (1932).
(212) Rosenberg, Paul: Phys. Rev. **55,** 1267 (1939); **57,** 561 (1940).
(213) Rosin, Seymour, and Rabi, I. I.: Phys. Rev. **48,** 373 (1935).
(214) Rostagni, A.: Nuovo cimento **11,** 34, 99 (1934); Z. Physik **88,** 55 (1934).
(215) Rudnick, Philip: Phys. Rev. **37,** 1707 (1931); **38,** 1342 (1931).
(216) Sasaki, N., and Nishibori, E.: Proc. Imp. Acad. (Tokyo) **12,** 10, 13 (1936); J. Chem. Soc. Japan **57,** 1277–99 (1936).
(217) Scheffers, H.: Physik. Z. **35,** 425 (1934).
(218) Scheffers, H.: Physik. Z. **37,** 220 (1936).
(219) Scheffers, H.: Physik. Z. **40,** 1 (1939).

(220) Scheffers, H.: Physik. Z. **41**, 89 (1940).
(221) Scheffers, H.: Physik. Z. **41**, 98 (1940).
(222) Scheffers, H., and Stark, J.: Physik. Z. **35**, 625 (1934).
(223) Scheffers, H., and Stark, J.: Physik. Z. **37**, 217 (1936).
(224) Schnurmann, Robert: Z. Physik **85**, 212 (1933); J. phys. radium **6**, 99 (1935).
(225) Schüler, H., and Korsching, H.: Z. Physik **103**, 434 (1936).
(226) Schüler, H., and Schmidt, Th.: Z. Physik **99**, 285 (1936).
(227) Schüler, H., and Schmidt, Th.: Z. Physik **104**, 468 (1937).
(228) Schwinger, Julian: Phys. Rev. **51**, 648 (1937).
(229) Shrader, E. F.: Phys. Rev. **58**, 475 (1940).
(230) Shrader, E. F., Millman, S., and Kusch, P.: Phys. Rev. **58**, 925 (1940).
(231) Simons, J. H., and Glasser, J.: J. Chem. Phys. **8**, 547 (1940).
(232) Simpson, O. C.: Dissertation, University of Illinois, 1934.
(233) Smyth, H. D.: Proc. Roy. Soc. (London) **A102**, 283 (1922).
(233a) Solomon, Jacques: Compt. rend. **202**, 1046 (1936).
(234) Starkey, T. V.: Phil. Mag. **18**, 241 (1934).
(235) Stern, O. Z. Physik **3**, 49, 417 (1920).
(236) Stern, O. Z. Physik **7**, 249 (1921).
(237) Stern, O. Physik. Z. **23**, 476 (1922).
(238) Stern, O. Z. Physik **39**, 751 (1926).
(239) Stern, O. Naturwissenschaften **17**, 391 (1929).
(240) Stern, O. Phys. Rev. **51**, 852 (1937).
(241) Stern, O. Phys. Rev. **51**, 1028 (1937).
(242) Stevenson, A. F.: Phys. Rev. **58**, 1061 (1940); **59**, 767 (1941).
(243) Taylor, J. B.: Z. Physik **57**, 242 (1929).
(244) Taylor, J. B.: Phys. Rev. **35**, 375 (1930).
(245) Torrey, H. C.: Phys. Rev. **51**, 501 (1937).
(246) Torrey, H. C.: Phys. Rev. **59**, 293 (1941).
(247) Torrey, H. C., and Spremulli, Paul F.: Work done at the Pennsylvania State College.
(248) Van Atta, C. M., and Granath, L. P.: Phys. Rev. **44**, 935 (1933).
(249) Van Vleck, J. H.: *The Theory of Electric and Magnetic Susceptibilities*, p. 266. Oxford University Press, London (1932).
(250) Varney, R. N.: Phys. Rev. **50**, 159 (1936).
(251) Veszi, G.: Z. physik. Chem. **B11**, 211 (1930).
(252) Veszi, G.: Z. physik. Chem. **B38**, 424 (1938).
(253) Wick, G. C.: Z. Physik **85**, 25 (1933).
(254) Wohlwill, M.: Z. Physik **80**, 67 (1933).
(255) Wood, R. W.: Phil. Mag. **30**, 300 (1915); **32**, 364 (1916).
(256) Wrede, Erwin: Z. Physik **44**, 261 (1927).
(257) Zabel, R. M.: Phys. Rev. **42**, 218 (1932).
(258) Zabel, R. M.: Phys. Rev. **46**, 411 (1934).
(259) Zacharias, J. R.: Phys. Rev. **60**, 168 (1941).
(260) Zacharias, J. R., and Kellogg, J. M. B.: Phys. Rev. **57**, 570 (1940).
(261) Zahl, Harold: Phys. Rev. **36**, 893 (1930).
(262) Zahl, H. A., and Ellett, A.: Phys. Rev. **38**, 977 (1931).
(263) Zartman, I. F.: Phys. Rev. **37**, 383 (1931).
(264) Young, L. A.: Phys. Rev. **42**, 218 (1932).

General references

Fraser, Ronald G. J.: *Molecular Rays*. University Press, Cambridge (1931).
Fraser, Ronald G. J.: *Molecular Beams*. The Chemical Publishing Company, Inc., New York (1938).

Review articles

BECKER, A.: Die Physik **6,** 55 (1938).
DALLAPORTA, NICOLA: Nuovo cimento **12,** 576 (1935). Magnetic moments.
FLEVORA, M. N.: Uspekhi Fiz. Nauk **15,** 614 (1935). Diffraction.
FRISCH, R., AND STERN, O.: *Handbuch der Physik,* Vol. XXII, 2, 351. Diffraction.
FUES, E.: *Handbuch der Experimentalphysik,* Ergänzungswerk Vol. II. Diffraction.
GERLACH, WALTHER: Ergeb. exakt. Naturw. **3,** 182 (1924).
GUILLEMIN, VICTOR, JR.: Rev. Sci. Instruments **5,** 411 (1934).
GUILLEMIN, VICTOR, JR.: J. Franklin Inst. **219,** 73 (1935).
KOPFERMANN, H.: Ergeb. exakt. Naturw. **15,** 229 (1936). Nuclear moments.
PERSICO, E.: Nuovo cimento **11,** 118 (1934).
RITSCHL, R.: Physik. Z. **38,** 141 (1937). Neutralization.
RODEBUSH, W. H.: Rev. Modern Phys. **3,** 392 (1931).
TAYLOR, J. B.: Ind. Eng. Chem. **23,** 1228 (1931).

ORGANOBISMUTH COMPOUNDS

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I. FOREWORD

The synthesis of triethylbismuth in 1850 by Löwig and Schweizer (76) inaugurated the study of the chemistry of organobismuth compounds. The spontaneous inflammability of these trialkyl derivatives limited investigation in the field until Michaelis and Polis (84) prepared triphenylbismuth in 1887. This aromatic compound was stable in air and could be converted to a pentavalent dichloride or dibromide which possessed unusual stability. In 1913, Challenger reported the beginning of an investigation on the preparation of optically active organobismuth compounds. In the years which followed, up until 1934, Challenger and his coworkers (13–27, 118), although they did not accomplish their original purpose, did make contributions of paramount importance in the field of organobismuth compounds.

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The effectiveness of triphenylbismuth, used as a salve, in the treatment of canine syphilis was reported by Giemsa (39, 40, 41) in 1923. Two years later, Supniewski and Adams (111) and Supniewski (109, 110) prepared a number of water-soluble organobismuth compounds for possible therapeutic utilization. Bismuth therapy in the past had been based entirely upon the use of colloidal bismuth, inorganic bismuth salts, or bismuth salts of organic acids. With these, in general, absorption by body fluids was slow and toxicity high.

The use of water-soluble organobismuth compounds offers new opportunities for investigation into bismuth therapy, but the synthesis of these compounds presents numerous difficulties. Organobismuth compounds undergo a limited number of reactions, and it is rarely possible, starting with known compounds, to prepare new derivatives possessing amino, carboxylic, or related groups. Of the many reactions reported, the synthesis of organobismuth compounds in liquid ammonia (52) and through the decomposition of aryldiazonium chloride–bismuth chloride complexes (53) appear to hold most promise.

II. The Chemistry of Organobismuth Compounds

A. INTRODUCTION

A study of the relative reactivities of organometallic compounds would indicate that reactivity, in part, is dependent upon the strength of the carbon–metal bond in the organometallic molecule. The organoalkali compounds which possess a weak and highly polar bond represent a highly reactive type. Hein and coworkers (65a) have demonstrated the highly polar character of these compounds by showing their ability to conduct an electric current when dissolved in the proper medium. The strength of the carbon–metal bond, and consequently reactivity, may be correlated also with the metallic nature of the central atom of the organometallic molecule, since with enhanced metallicity the bond becomes increasingly weaker. It has been predicted, therefore, on the basis of these generalizations, that Element 87 will form the most reactive organometallic compounds.

In their thorough investigation of the triphenyl compounds of the elements of the B family of Group V, Smith and Andrews (106) have observed a definite increase in molal heat capacity with increasing atomic weight of the central atom. As an explanation of this they have suggested the possibility that the greater atomic volume of the element would give the R groups attached to it increased freedom of motion and hence less stability. It should be noted, however, that their data, numerically, give little indication of the difference in stability and reactivity between two adjacent members of this family, e.g., antimony and bismuth.

It has been observed that the relative reactivities of many RM compounds parallel and may be related directly to the ionization potentials of the corresponding metallic atoms (45a). In a given group or subgroup, the lower the ionization potential of the metal the more reactive will be its simple organometallic compound. There appear to be some exceptions but, by and large, this generalization holds true. The ionization potentials for bismuth and antimony are not known with certainty, but are given as 8 and 9 volts, respectively. This difference of approximately 1 volt is large when it is seen that the difference between the potentials of lithium and cesium is only 1.49 volts. Thus the ionization potentials of the corresponding gaseous metallic atoms would indicate marked distinctions between the reactivities of organoantimony and organobismuth compounds, and this is actually the case. The carbon–antimony linkage is extremely stable, whereas the weakness of the carbon–bismuth bond is the most characteristic property of organobismuth compounds.

Organobismuth compounds resemble organomercury, organolead, and organotin compounds in reactivity. Cleavage reactions with hydrogen chloride and trichloroacetic acid,

$$(C_6H_5)_3Bi + \left\{\begin{array}{ll} HO_2CCCl_3 & \rightarrow C_6H_6 + (C_6H_5)_2BiO_2CCCl_3 \\ HCl & \rightarrow C_6H_6 + (C_6H_5)_2BiCl \end{array}\right.$$

would indicate that tetraphenyllead and diphenylmercury are more reactive than triphenylbismuth and that tetraphenyltin is extremely unreactive (12). Cleavage with formic and acetic acids, however, would give triphenylbismuth a greater reactivity than diphenylmercury or tetraphenyllead (71). Again, diphenylmercury and bismuth bromide yield triphenylbismuth in quantitative yields (18), whereas trivalent or pentavalent aromatic bismuth compounds and mercuric chloride do not form R_2Hg compounds (77). Thus, organomercury compounds might be considered more reactive than organobismuth compounds, since the preparation of a less reactive organometallic compound is accomplished, in general, by the reaction between a more reactive organometallic compound and a metal halide. The reaction between diphenylmercury and bismuth bromide is not a reaction of general application, however, since di-*p*-tolylmercury, di-*p*-bromophenylmercury, di-*o*-carbomethoxyphenylmercury, or di-*p*-carboethoxyphenylmercury and bismuth bromide do not yield organobismuth compounds (55). A similar behavior has been noted also by Michaelis and Reese (85, 86) and by Hasenbäumer (64) in the reaction between diphenylmercury and antimony chloride. The former authors could not obtain organoantimony compounds by the fusion of diphenylmercury and antimony chloride alone, or in the presence of solvents, whereas the latter obtained traces of triphenylantimony dichloride and diphenylantimony trichloride from the same reactants in dry xylene at 130°C. in a sealed tube.

Hilpert and Grüttner (67) have reported that diphenylmercury and bismuth metal at 250°C. in a stream of hydrogen gave a 41 per cent yield of triphenylbismuth, while triphenylbismuth and mercury under the same conditions gave but a 25 per cent yield of diphenylmercury. The yield of triphenylbismuth in the first experiment may have been higher, since organobismuth compounds are cleaved by hydrogen (68). The bismuth compound was not isolated, but the residue remaining from the xylene extraction of the reaction mixture was analyzed for bismuth. On this basis, organobismuth compounds might be considered more reactive than organomercury compounds. It has not been possible to extend this reaction, since di-*p*-tolylmercury and bismuth metal at 233°C. yield only free mercury and toluene. Both di-*p*-tolylmercury and tri-*p*-tolylbismuth are pyrolyzed even at the boiling point of *p*-cymene. No pyrolysis occurs in benzene or xylene, and at these temperatures di-*p*-tolylmercury and bismuth metal do not react. With mercury, neither tri-*p*-tolylbismuth nor triphenylbismuth yields organomercury compounds when refluxed in benzene (55), but under the same conditions triphenylthallium and diphenylcadmium react with mercury to form diphenylmercury (45a). On the basis of cleavage reactions with *n*-butylmercaptan and selenophenol, Nelson (47, 89) has shown organomercury compounds to be less reactive than both organobismuth and organolead compounds. Further complications are introduced by cleavage reactions with thiophenol which indicate the order Hg > Bi > Pb > Sn (55.)

As Nelson has pointed out, the reactivity of organolead, organobismuth, and organomercury compounds toward the —SH and —SeH groups is anomalous, since these compounds are cleaved more readily by thiols and selenols than by more acidic carboxylic acids. He has suggested that a reactivity series based on cleavage by acidic hydrogens exclude those hydrogens of the selenol and thiol types. It is probable, too, that the tellurols would show the same anomalous behavior.

On the basis of the cleavage reactions with the carboxylic acids, Nelson has proposed the order of reactivity Pb > Hg > Bi > Sn, and for cleavage by —SH and —SeH compounds, the order Bi > Pb > Hg > Sn. In all probability his first series is the more accurate one, although it is the reverse of the Koton series, Bi > Hg > Pb, which is based on cleavage by carboxylic acids also.

It is very apparent, because of these conflicting data, that the term "relative reactivities" when applied to a series of organometallic compounds must be based on *true* equilibrium reactions which have been allowed to come to equilibrium. This is very difficult with organometallic compounds, where solubility, solvent, instability, and diverse unknown but significant factors seriously limit the number of suitable reactions. Of the reactions described above, only one appears to have a real significance. Hilpert and Grüttner (67) made an attempt to approach the true equilibrium between triphenylbismuth and mercury, and diphenylmercury and bismuth. In the other reactions all that has been measured is the *rate* at which a reaction is proceeding.

Although compounds containing >As—As< and >Sb—Sb< groups have been prepared, Paneth and Loleit (93) mention only the possible formation of bisdimethylbismuth, $(CH_3)_2BiBi(CH_3)_2$, and bisdiethylbismuth, $(C_2H_5)_2BiBi(C_2H_5)_2$, in the reactions between heated bismuth mirrors and free methyl and ethyl radicals.[2] The dimethylbismuth reported by Denham (32) may have been bisdimethylbismuth. Ganassini and Santi (38) have prepared this compound or its oxide, $(CH_3)_2BiOBi(CH_3)_2$, by the reaction between sodium acetate and bismuth oxide. Blicke, Oakdale, and Smith (7) and Rozenblumówna and Weil (100) were unable to prepare bisdiphenylbismuth by treating diphenylbismuth halides with various reducing agents. The probable formation of the diarylbismuth radical in liquid ammonia has been reported recently (52). For example, di-*p*-tolylbismuth halide and one atom of sodium yield an intensely green colored solution.

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[2] F. O. Rice and A. L. Glasebrook (J. Am. Chem. Soc. **56**, 2381 (1934)) have found that the methylene radical, prepared by the thermal decomposition of diazomethane, will not remove bismuth mirrors. Antimony and arsenic mirrors, however, are removed.

$$(p\text{-}CH_3C_6H_4)_2BiX + Na \rightarrow (p\text{-}CH_3C_6H_4)_2Bi + NaX$$

The addition of a second atom of the metal replaces the green color by a dark red color which is characteristic of diarylbismuth sodium compounds.

$$(p\text{-}CH_3C_6H_4)_2Bi + Na \rightarrow (p\text{-}CH_3C_6H_4)_2BiNa$$

The first reaction has been used to prepare a variety of free radicals in liquid ammonia (44).

Compounds containing —As═As— and —Sb═Sb— groups are known, but the corresponding bismuth compounds have never been prepared. Bismuth compounds with mixed metal-metal bonds, e.g., —Bi═As—, are unstable, easily oxidized by air, and decomposed by boiling water (35). Triphenylbismuth dichloride and copper bronze do not yield $(C_6H_5)_3Bi{=}Bi(C_6H_5)_3$, even though pentavalent bismuth compounds like $(Aryl)_3BiX_2$ possess unusual stability (19). Phenylbismuth dibromide and copper bronze yield only triphenylbismuth (18).

No bismuth compounds analogous to the aromatic arsonic and stibonic acids, $RAsO(OH)_2$ and $RSbO(OH)_2$, have been prepared. Actually, H_3BiO_3 is a moderately strong base, and basic bismuth salts of these acids have been made and investigated as spirocheticidal and treponemicidal agents. Lecoq (74) has reported the preparation of phenylbismuth oxide, C_6H_5BiO, which may hydrate to a pentavalent form, but this is questionable. Triarylbismuth dihydroxides, $R_3Bi(OH)_2$, have been prepared (22, 55) and are easily soluble in water.

$$R_3BiCl_2 + 2AgOH \rightarrow R_3Bi(OH)_2 + 2AgCl$$

The pH of such a solution is approximately 7.2, and after removal of the silver chloride by centrifuging, the solution may be used therapeutically (55). The aqueous solution undergoes gradual decomposition, particularly in the presence of light. By evaporation of the aqueous solution the dihydroxide may be obtained as a pale yellow powder which does not redissolve in water but dissolves readily in 95 per cent ethanol. Ethanolic solutions also undergo decomposition, yielding acetaldehyde and bismuth hydroxide.

Primary and secondary arsines, $RAsH_2$ and R_2AsH, where R is aliphatic or aromatic, are known, but the corresponding antimony or bismuth compounds have not been prepared.

Tertiary aromatic and aliphatic arsenic compounds and tertiary alkyl- and some mixed alkylaryl-antimony compounds add alkyl halides to form "onium" compounds. Tertiary bismuth compounds, both aliphatic and aromatic, do not add alkyl halides even at elevated temperatures. Generally, a secondary cleavage reaction takes place.

$$(CH_3)_3Bi + 2CH_3I \xrightarrow{200°C.} CH_3BiI_2 + 2C_2H_6$$

The reaction between triphenylbismuth dibromide and ethylmagnesium bromide might have yielded a bismuthonium compound.

$$(C_6H_5)_3BiBr_2 + C_2H_5MgBr \rightarrow (C_6H_5)_3(C_2H_5)BiBr + MgBr_2$$

The products, instead, were triphenylbismuth, diphenylbismuth bromide, and bromobenzene (14). It cannot be assumed that ethyltriphenylbismuthonium bromide was formed as an intermediate and decomposed in a manner similar to that which takes place when trimethylphenylarsonium iodide is heated (119),

$$(CH_3)_3(C_6H_5)AsI \rightarrow (CH_3)_2(C_6H_5)As + CH_3I$$

since no onium compound was formed when triphenylarsenic dihalide was treated with methylmagnesium iodide. There occurred, instead, reduction to triphenylarsenic.

The most stable valence in organobismuth compounds is 5. This valence is not demonstrated by the trialkylbismuth compounds, although it has been proposed that pentavalent derivatives are formed as intermediates and decompose spontaneously (80). The pentavalent aromatic bismuth compounds are comparatively stable. The various derivatives with halogens and pseudohalogens show a regular decrease in stability, the difluoride being the most stable. On the basis of decomposition temperatures, these derivatives, in the order of decreasing stability are: difluoride, dichloride, dicyanate, dibromide, diazide, and diiodide. The dithiocyanate and diselenocyanate have not been prepared.

Birchenbach and Kellermann (6) have determined the decomposition potentials of a large number of potassium halides and pseudohalides. Since the decomposition potential is related directly to stability, it was possible to obtain a series of halogens and pseudohalogens based on the relative ease of decomposition. This series,—F, CNO, OCN, Cl, N_3, Br, CN, SCN, I, SeCN, TeCN,—agrees well with the series described above.

Trivalent bismuth compounds are cleaved quantitatively by inorganic acids. In contrast, the pentavalent compounds are but little affected by fuming nitric acid, boiling hydrochloric acid, or sulfuric acid, except for nuclear substitution. This stability is demonstrated by the following reactions:

$$(C_6H_5)_3Bi(NO_3)_2 \xrightarrow{\text{concd. HCl}} (C_6H_5)_3BiCl_2$$

$$(C_6H_5)_3Bi(NO_3)_2 \xrightarrow{\text{fuming } HNO_3} (O_2NC_6H_4)_3Bi(NO_3)_2$$

$$(C_6H_5)_3BiCl_2 \xrightarrow{\text{concd. } H_2SO_4} (C_6H_5)_3BiSO_4$$

B. GENERAL METHODS FOR THE PREPARATION OF TERTIARY BISMUTH COMPOUNDS

Organobismuth compounds are best prepared by interaction of a Grignard reagent and bismuth chloride. The yields range from 60 to 80 per cent with most aromatic bismuth compounds. Triethylbismuth has been obtained in 86 per cent yield by this method (47). It has not been possible to introduce bismuth into the aromatic nucleus by means of the Bart reaction (3) or its modifications (26, 111). The synthesis of organobismuth compounds by the copper bronze decomposition of aryldiazonium chloride–bismuth chloride complexes has been described recently (48, 53).

Tertiary bismuth compounds have been prepared by the following methods:

(*a*) *Reaction of a bismuth–sodium or bismuth–potassium alloy with an alkyl or aryl halide:* Löwig and Schweizer (76) synthesized triethylbismuth by this method in 1850. This was the first organobismuth compound reported in the literature. The reaction may be represented as follows:

$$3C_2H_5X + BiK_3 \rightarrow (C_2H_5)_3Bi + 3KX$$

Breed (8) and Dünhaupt (34) also prepared triethylbismuth, using a bismuth–potassium alloy. Michaelis and Polis (84), Michaelis and Marquardt (83), and Gillmeister (43) obtained aromatic bismuth compounds by means of a bismuth–sodium alloy and an aryl halide. Rozenblumówna and Weil (100) have reported more recently a modification of the Gillmeister method which gave improved yields. Stilp (108) has prepared triphenylbismuth in 75 per cent yield by the Michaelis method.

(*b*) *Reaction between aluminum carbide and bismuth chloride in hydrochloric acid:* Hilpert and Ditmar (66) obtained trimethylbismuth by the slow addition of aluminum carbide to bismuth chloride in 20 per cent hydrochloric acid solution.

$$2Al_4C_3 + 2BiCl_3 + 18HCl \rightarrow 2(CH_3)_3Bi + 8AlCl_3$$

It is apparent that this reaction is limited to the preparation of trimethylbismuth and that the yield would be affected by acid cleavage.

(*c*) *Reaction of a dialkylzinc compound and a bismuth halide:* Marquardt (80, 81) prepared trialkylbismuth compounds by the addition of an ether solution of a bismuth halide to a similar solution of a dialkylzinc compound. The difficulty in handling the spontaneously inflammable alkylzinc compounds has made it necessary to replace this method by the equally effective but more easily handled Grignard reagent.

(*d*) *Reaction of the Grignard reagent and a bismuth halide:* Pfeiffer and Pietsch (97) were the first to prepare organobismuth compounds by means of the Grignard reagent. This method was later employed by Stilp (108), Challenger and coworkers (14, 15, 18, 23), Worrall (120), Supniewski and Adams (111), Supniewski (109, 110), Classen and Ney (29), Krause and Renwanz (72), Blicke, Oakdale, and Smith (7), Davies, Norvick, and Jones (31), Gilman and coworkers (47, 51, 54), Grüttner (62, 63), Fr. Fabrikant (37), and Zhitkova and coworkers (121). With phenyllithium and bismuth bromide, the yield of triphenylbismuth is

44 per cent (121); this method is therefore inferior to the Grignard method, which gives yields of about 80 per cent. It should be noted that, regardless of the quantity of bismuth halide used, except when it exceeds the quantity demanded by the equation

$$3RMgX + BiX_3 \rightarrow R_3Bi + 3MgX_2$$

the tertiary compound is formed. The reaction can be used to prepare $RBiX_2$ and R_2BiX compounds but not in good yields (63, 74, 114).

(*f*) *Reaction between diphenylmercury and bismuth metal:* Hilpert and Grüttner (67) have reported a 41 per cent yield of triphenylbismuth when bismuth metal and diphenylmercury are heated at 250°C. Frankland and Duppa (36) prepared triethylbismuth by heating diethylmercury and powdered bismuth at 100–130°C. Tetraethyllead and powdered bismuth do not react after 5 hr. heating at 100–130°C. Tetraethyllead and bismuth chloride yield a mixture of ethylbismuth chlorides and probably some triethylbismuth (50). Phenylmercuric chloride and bismuth–sodium alloy yield traces of triphenylbismuth (121).

(*g*) *Reaction between bismuth electrodes and a gaseous hydrocarbon under the influence of an electrical discharge:* Trimethylbismuth is formed by an electrical discharge between two bismuth electrodes if a stream of methane is passed between the electrodes simultaneously with the electrical discharge (92).

(*h*) *Reaction between free radicals and bismuth mirrors:* Paneth and Hofeditz (92), Paneth (94), and Rice and coworkers (98) have reported that free methyl and ethyl radicals dissolve bismuth mirrors. Paneth and Loleit (93) have obtained trialkylbismuth compounds with cold mirrors and methyl and ethyl radicals. With heated mirrors, in addition to the trialkyl compounds, there was evidence of the possible formation of bisdimethylbismuth and bisdiethylbismuth.

(*i*) *Spontaneous decomposition of diphenylbismuth sodium in liquid ammonia:* The spontaneous decomposition of diphenylbismuth sodium in liquid ammonia leads to the formation of triphenylbismuth in 49 per cent yield (52). Sodium–bismuth alloy and iodobenzene do not form triphenylbismuth in liquid ammonia, owing probably to the insolubility of the alloy in this solvent (52).

(*j*) *Decomposition of an aryldiazonium chloride–bismuth chloride complex, followed by treatment with ammonia or hydrazine:* The recently described preparation of triarylbismuth compounds by the decomposition of an aryldiazonium chloride–bismuth chloride complex by copper, followed by treatment with ammonia (48), has unusual possibilities for the synthesis of therapeutically active organobismuth compounds. Thus far, however, it has not been possible to obtain experimental conditions favorable to the preparation of compounds like tri-*p*-carboxyphenylbismuth or tri-*p*-sulfophenylbismuth. In any case the reaction has some advantages, since it has made possible the preparation of di-*o*-carbomethoxyphenylbismuth chloride, di-*o*-carboethoxyphenylbismuth chloride, and *o*-carbomethoxyphenylbismuth dichloride, which could not have been prepared so readily by other methods. It affords a simplified procedure, also, for the preparation of tri-*p*-bromophenylbismuth (53).

The mechanism of this reaction has been established by isolating the intermediary $RBiX_2$ and R_2BiX compounds formed. It has been found, in addition, that hydrazine is a reagent of choice for the conversion of these intermediates to R_3Bi compounds.

$$(C_6H_5N_2Cl)_2 \cdot BiCl_3 + 4Cu \longrightarrow (C_6H_5)_2BiCl + 4CuCl + 2N_2$$

$$(C_6H_5)_2BiCl \xrightarrow{H_2NNH_2} (C_6H_5)_3Bi$$

(*k*) *Reaction between an* R_2BiX *or* $RBiX_2$ *compound and the Grignard reagent:* This reaction was first used by Challenger (14) to prepare diphenyl-α-naphthylbismuth, and later by Norvick (90) to prepare diethylamylbismuth. A modified procedure employing low temperatures has been utilized successfully in the preparation of a large number of related $R_2R'Bi$ compounds (51).

(*l*) *Reaction between a diarylbismuth sodium compound and an aryl iodide or bromide:* Unsymmetrical organobismuth compounds have been prepared by the reaction of an R_2BiNa compound and α-iodonaphthalene in liquid ammonia. Some aryl bromides react also, but substituents ortho to the halogen inhibit reaction (52).

$$(C_6H_5)_2BiNa + \alpha\text{-}C_{10}H_7I \rightarrow (C_6H_5)_2(\alpha\text{-}C_{10}H_7)Bi + NaI$$

(*m*) *Conversion of triarylbismuth dihalides to triarylbismuth compounds:* Tri-*m*-tolylbismuth, because of its low melting point, is best purified as the pentavalent dichloride (111). Diphenyl-α-naphthylbismuth dichloride may be separated from triphenylbismuth dichloride by virtue of the insolubility of the latter compound in acetone (20). Impure oily unsymmetrical bismuth compounds have been converted to their dichlorides in order to facilitate purification (51). These halogens may be removed by hydrogen sulfide (84), ammonium sulfide (83), moist silver oxide (19, 43), sodium hydrosulfite, formaldehyde, sodium and alcohol, and dimethyl sulfate and alkali (111). Hydrazine hydrate is a reagent of choice for this reaction (49).

(*n*) *Conversion of arylbismuth dihalides and diarylbismuth halides to triarylbismuth compounds:* Hydrazine hydrate converts $RBiX_2$ and R_2BiX compounds to R_3Bi in excellent yields. Alcoholic ammonia has been used also, but no data are available as to its effectiveness (23, 83).

(*o*) *Formation of trimethylbismuth by the spontaneous disintegration of tetramethylradium D:* Mortensen and Leighton (87) have investigated the molecular changes accompanying radioactive transformations by measuring the β-activity of tetramethyllead containing small quantities of tetramethylradium D. These authors were interested in determining whether the molecule was completely decomposed, or whether it adjusted itself to the new valence conditions. The transformation of radium D into radium E and then into radium F is essentially the change of an atom of lead into bismuth and then into polonium. Radium D emits only β-rays which are too feeble to produce an appreciable effect upon an electroscope, while radium E and radium F possess strong α- and β-ray activity. By the examination of the vapor above the radioactive tetramethyllead, these authors found indisputable evidence for the presence of radium E (presumably as trimethylbismuth) and radium F (presumably as dimethylpolonium). From this they concluded that the tetramethylradium D molecule was not completely broken down by radioactive transformation, but adjusted itself to the new valence conditions by the loss of a methyl group and the completion of the electron pair by the acquisition of an electron from its surroundings. The disintegration apparently involves the rupture of one bond only.

(*p*) *Formation of organobismuth compounds by metal–metal interchange:* Leigh-Smith and Richardson (75) have obtained evidence which suggests that bismuth metal, deposited presumably as the oxide on a metallic surface, can exchange with lead in tetramethyllead to form trimethylbismuth. This they demonstrated in the following manner:

A piece of gold which had been activated with Th(B + C + C′ + C″) was immersed in an ether solution of tetramethyllead for 2 hr. Then a small quantity of the solution was introduced into a small pot inside a Wilson expansion chamber filled with nitrogen. As the solution evaporated from the pot, characteristic tracks of Th(C + C′) were observed starting from parts of the chamber remote from the pot, thus proving that a volatile compound had been formed which evaporated with the rest of the solution, and this compound, presumably, is trimethylbismuth. This exchange process occurs apparently between atoms of the same atomic number and without the breaking up of the molecule.

The reaction between *n*-butyllithium or *n*-butylsodium and triarylbismuth compound does not lead to the expected metalation of the aromatic nucleus (54). Instead, a metal–metal interconversion takes place.

$$(p\text{-}CH_3C_6H_4)_3Bi + 3n\text{-}C_4H_9Li \rightarrow (n\text{-}C_4H_9)_3Bi + 3p\text{-}CH_3C_6H_4Li$$

Phenyllithium and bismuth metal do not yield triphenylbismuth, whereas phenyllithium and antimony give triphenylantimony (113). This again demonstrates the sharp differences between antimony and bismuth. Many reactions which can be used to prepare organoantimony compounds in good yields fail entirely in the preparation of organobismuth compounds.

(*q*) *Reaction between* R_3Bi *and* RX *compounds:* Marquardt (81) has reported that, in the course of heating triisobutylbismuth with methyl or ethyl iodide at 150°C., he obtained no quaternary iodides but, instead, trimethylbismuth and triethylbismuth, respectively.

(*r*) *Electrolysis of ethylsodium in diethylzinc, using bismuth as the anode:* The electrolysis of an ethylsodium–diethylzinc solution probably gives free ethyl radicals. Triethylbismuth was obtained when the electrolysis was carried out with bismuth as the anode (65b).

C. ORGANOBISMUTH COMPOUNDS

1. *Symmetrical trialkylbismuth compounds*

The trialkylbismuth compounds are heavy, highly refractive, colorless or pale yellow, oily liquids. Tricyclohexylbismuth appears to be a solid at room temperature. The methyl and ethyl compounds possess unpleasant odors, but the higher homologs are odorless. Their vapors irritate mucous membranes. Because of their inflammability in air they must be isolated under an inert atmosphere, and with the exception of the trimethyl compound they cannot be distilled at ordinary pressures without decomposition. Triethylbismuth, for example, explodes when heated to 150°C. (8, 34). Dünhaupt (34) has reported that the presence of water or ether makes possible a distillation of triethylbismuth at atmospheric pressure. Seifter (105) has found that it is possible to distil a trialkylbismuth-etherate without decomposition. Triethylbismuth becomes cloudy when exposed to atmospheric oxygen, and this clouding effect has been proposed as a delicate test for the presence of oxygen (47).

Moureu, Dufraisse, and Badoche (88) have shown that trialkylbismuth compounds can function both as catalyst and inhibitor in the autoöxidation of organic compounds. Triethylbismuth effectively retards the oxidation of benzaldehyde, furfural, butyraldehyde, and alkaline solutions of sodium sulfite but accelerates the oxidation of styrene, turpentine, and acid solutions of sodium sulfite.

The boiling points of the trialkylbismuth compounds increase normally, and the branched-chain compounds have lower boiling points than the normal isomers. Their specific gravities, as one might predict, decrease with increasing carbon content. They are soluble in alcohol, ether, petroleum ether, carbon tetrachloride, and benzene, and are insoluble in water.

Trialkylantimony compounds combine readily with oxygen, but the ease of oxidation decreases with increasing molecular weight. They ignite when warmed in air, with the formation of antimony trioxide. Trimethylantimony oxide, triethylantimony oxide, and triamylantimony oxide can be prepared directly by the regulated oxidation of the respective tertiary compounds. With chlorine, bromine, iodine, cyanogen bromide, cyanogen iodide, sulfur, and selenium, the trialkylantimony compounds form stable pentavalent compounds. They also form double salts with platinum and palladium chlorides. Mercuric, auric, and silver salts are reduced to the free metal. With alkyl halides they readily form stibonium halides. They dissolve in nitric acid to form dinitrates.

The trialkylbismuth compounds, in contrast, form no stable pentavalent derivatives. With the exception of the trimethyl compound, which is the most stable of the trialkylbismuth compounds, they ignite spontaneously and burn to bismuth oxide, although the ease of oxidation decreases with increasing molecular weight. In the presence of pure oxygen, tributylbismuth explodes violently (54).[3] With chlorine or bromine, trialkylbismuth compounds undergo cleavage even at 0°C. to form R_2BiX compounds. Inorganic acids cleave the bismuth compounds and with concentrated nitric and sulfuric acids the reaction proceeds explosively. With alkyl iodides, quaternary bismuthonium iodides do not form even at elevated temperatures. Hydrogen sulfide cleaves the bismuth compounds. Both silver nitrate and mercuric chloride readily cleave these compounds and are themselves reduced to the free metal.

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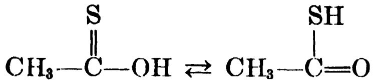
[3] G. Calingaert, H. Soroos, and V. Hnizda (J. Am. Chem. Soc. **64**, 392 (1942)) have found that the oxidation of triethylbismuth with pure oxygen under controlled temperature yielded bismuth oxide, diethylbismuth ethoxide, ethoxybismuth oxide, diethyl peroxide, diethyl ether, ethyl alcohol, and ethylene.

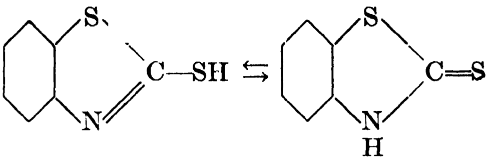
It has been reported that triethylbismuth may be used for the detection of the —SH group (47). The test is based on the cleavage of triethylbismuth by acid hydrogens,

$$(C_2H_5)_3Bi + R{-}{-}{-}H \rightarrow C_2H_6 + (C_2H_5)_2Bi{-}{-}{-}R$$

and is carried out using the ordinary Zerewitinoff technique. No cleavage occurs with the hydrogens in —NH, —C≡CH, or simple —OH groups, although some strong carboxylic acids give limited reactions. There is no interference by azo or nitro groups. The existence of thioenolization in thioacetic acid and 1-mercaptobenzothiazole, for example, is shown quite definitely.



$$CH_3{-}\overset{\overset{\displaystyle S}{\|}}{C}{-}OH \rightleftarrows CH_3{-}\overset{\overset{\displaystyle SH}{|}}{C}{=}O$$



2. *Symmetrical triarylbismuth compounds*

The tertiary aromatic bismuth compounds are colorless crystalline solids, unaffected by oxygen and possessing generally a characteristic pleasant odor. They are soluble in ethanol, ether, chloroform, ethyl acetate, acetone, petroleum ether, dioxane, and benzene and are insoluble in water. Triphenylbismuth may be distilled or sublimed without decomposition at very low pressures and can be obtained so pure that it has been used in determinations of the atomic weight of bismuth (28, 29, 30). The molecular weights of triphenylbismuth, tri-α-naphthylbismuth, and tri-*p*-tolylbismuth determined cryoscopically in benzene are normal. This is further evidence that even the more stable aromatic compounds show no tendency to form bismuth–bismuth bonds,

$$\geqslant Bi{=}Bi \leqslant$$

even in view of their greater stability when the maximum valence has been satisfied. The aromatic groups are held loosely by bismuth and are cleaved readily by inorganic and organic acids, thiophenol, hydrogen, and inorganic halides.

With either chlorine or bromine, tertiary aromatic bismuth compounds form dichlorides and dibromides (R_3BiX_2), respectively. Triphenylbismuth diiodide is stable only at −78°C. It is formed by titrating triphenylbismuth at this temperature with iodine, using starch as the indicator. The end point occurs when exactly two equivalents of iodine have been added. Raising the temperature but a few degrees causes a spontaneous decomposition. If the temperature is kept at −78°C., however, the diiodide may be kept unchanged for days. The difluoride is prepared by a double decomposition reaction between the dichloride and potassium fluoride.

With sulfuryl chloride, sulfur monochloride, and thionyl chloride tertiary compounds yield dichlorides (15, 18). With the halogen halides or halogen pseudohalides,—iodine chloride, iodine bromide, cyanogen chloride, etc.,—the tertiary compounds are cleaved. The more negative group or atom attaches itself to the bismuth, while the more positive group or atom is eliminated as part of the aromatic nucleus (17, 18, 19, 118). Such reactions give further confirmation to the stability series of halogens and pseudohalogens mentioned previously (page 12).

$$(C_6H_5)_3Bi + \left\{\begin{array}{llll} ICl & \longrightarrow & (C_6H_5)_2BiCl & + C_6H_5I \\ CNI & \longrightarrow & (C_6H_5)_2BiCN & + C_6H_5I \\ CNBr & \longrightarrow & (C_6H_5)_2BiBr & + C_6H_5CN \\ IBr & \longrightarrow & (C_6H_5)_2BiBr & + C_6H_5I \end{array}\right.$$

The ease with which bismuth exchanges its aromatic groups for the halogens of inorganic halides is evidence again of the weak carbon-bismuth bond. Under conditions where triphenylantimony reacts to form R_3SbX_2 compounds, triphenylbismuth cleaves to yield R_2BiX and $RBiX_2$ compounds (21, 23, 57, 59).

$$(C_6H_5)_3Bi + \left\{\begin{array}{llll} BiCl_3 & \rightarrow & (C_6H_5)_2BiCl & + C_6H_5BiCl_2 \\ SbCl_3 & \rightarrow & (C_6H_5)_2BiCl & + (C_6H_5)_3SbCl_2 \\ AsCl_3 & \rightarrow & (C_6H_5)_2BiCl & + C_6H_5AsCl_2 \\ PCl_3 & \rightarrow & (C_6H_5)_2BiCl & + C_6H_5PCl_2 \\ SiCl_4 & \rightarrow & (C_6H_5)_2BiCl & + \quad - - - \\ CuCl_2 & \rightarrow & (C_6H_5)_2BiCl & + \quad - - - \\ TiCl_4 & \rightarrow & (C_6H_5)_2BiCl & + \quad - - - \\ HgCl_2 & \rightarrow & (C_6H_5)_2BiCl & + C_6H_5HgCl \\ TlCl_3 & \rightarrow & (C_6H_5)_2BiCl & + (C_6H_5)_2TlCl \\ AgNO_3 & \rightarrow & \text{Probably } (C_6H_5Ag)_2 \cdot AgNO_3 & \end{array}\right.$$

The reaction with silicon tetrachloride yields an unidentified organosilicon compound; with cupric chloride there was no mention of the possible formation of organocopper compounds. No reduction to trivalent titanium occurs in the reaction with titanium tetrachloride. Considerable reduction occurs in the interaction of a Grignard reagent and titanium tetrachloride.

Stilp (108) has been the only one to report the formation of double salts in the reaction between tertiary aromatic bismuth compounds and mercuric chloride. Gillmeister (43) had reported previously that no double salt was formed between triphenylbismuth and mercuric chloride. Stilp did not prepare this salt but he was able to form others: $(p\text{-}CH_3C_6H_4)_3Bi \cdot HgCl_2$, $(o\text{-}CH_3C_6H_4)_3Bi \cdot HgCl_2$, $(m\text{-}(CH_3)_2C_6H_3)_3Bi \cdot HgCl_2$, and $(\alpha\text{-}C_{10}H_7)_3Bi \cdot HgCl_2$. The preparation of the tri-*p*-tolylbismuth double salt has been confirmed (55). These compounds are colorless crystalline solids which can be recrystallized without decomposition from glacial acetic acid. Compounds of this type could be useful as derivatives, since they possess sharp melting points and are easily purified.

Challenger and Pritchard (21) have found that migration of phenyl groups from bismuth may be effected either to the comparatively electropositive tin and mercury or to electronegative elements like arsenic and phosphorus. It was earlier proposed by Hilpert and Grüttner (67) that such migration took place only from the less positive to the more positive element, although the presence of halogens appeared to modify the reaction. The former authors consider that many of the ready migrations of phenyl groups are partly conditioned by the insolubility of one of the possible products.

With triphenylantimony, migration of phenyl groups occurs very rarely at ordinary temperatures. It has been observed with silicon tetrachloride, mercuric chloride, phosphorus trichloride, and with antimony trichloride only at 240°C. Addition compounds are produced almost always with triphenylarsenic. This is further evidence of the stability of the bond between carbon and the more electronegative elements.

Organobismuth compounds do not add to benzaldehyde, benzalacetophenone (55), or Michler's ketone (79). They do react, however, to a limited extent with acid halides (23) to give ketones in small yields. Under similar conditions, triphenylantimony does not react with acid halides. With chloroform in a sealed tube at 160°C. triphenylbismuth forms small quantities of triphenylmethane (26), and with benzyl chloride it forms diphenylmethane (23).

Triphenylbismuth reacts with benzoyl peroxide to form triphenylbismuth dibenzoate, $(C_6H_5)_3Bi(O_2CC_6H_5)_2$ (27), and with benzoyl nitrate to form triphenylbismuth dinitrate (118). It has been reported that triphenylbismuth gives color reactions with certain nitro derivatives (15), but no details are available.

Tribenzylbismuth oxidizes spontaneously when exposed to air, yielding benzaldehyde and inorganic bismuth (23). Tri-β-phenylethylbismuth undergoes a similar oxidation (55). This may account for the reported failure to prepare tri-*p*-methoxybenzylbismuth and tri-*o*-methoxybenzylbismuth (111). It has been reported also that bromophenacetin, *p*-chlorophenol, and *o*-iodoaniline with sodium–bismuth alloy do not yield tertiary bismuth compounds (100).

Makarova and Nesmeyanov (78) have prepared benzenediazonium nitrate by the reaction between triphenylbismuth, nitric oxide, and nitrogen trioxide. Supniewski and Adams (111) were unable to obtain triarylbismuth compounds possessing amino groups by the reduction of trinitroarylbismuth compounds. Unstable mixtures were the only products. Tri-*p*-dimethylaminophenylbismuth has been prepared by the interaction of *p*-dimethylaminophenyllithium and bismuth chloride, but this compound was cleaved by acetic acid in preference to forming a water-soluble acetate. It was cleaved also by chlorine even at 0°C., so that the preparation of a more stable pentavalent derivative was not possible (51).

Challenger, Peters, and Halévy (20) have attempted the preparation of triphenylbismuth diselenocyanate, $(C_6H_5)_3Bi(SeCN)_2$, by the interaction of triphenylbismuth dichloride and potassium selenocyanate (KSeCN). They obtained, instead, the unstable diphenylbismuth selenocyanate, $(C_6H_5)_2BiSeCN$. Tri-*p*-tolylbismuth dichloride and potassium selenocyanate underwent a similar reaction. The instability of the pentavalent compound was to be predicted. Triphenylbismuth and tellurium dicyanide, $Te(CN)_2$, gave diphenylbismuth cyanide.

When triarylbismuth compounds are heated to high temperatures, they

TABLE 1

*Tertiary bismuth compounds**

AROMATIC COMPOUNDS	MELTING POINT °C.	REFERENCES
Tri-*o*-anisyl-	171	(108, 110, 111)
Tri-*p*-anisyl-	190	(43, 108, 110)
Tribenzyl-	85	(13, 26, 55)
Tribiphenyl-	183	(120)
Tri-*p*-bromophenyl-	149	(23, 53)
Tri-*p*-carboxyphenyl-	162	(110)
Tri-*o*-chlorophenyl-	141	(51)
Tri-*p*-chlorophenyl-	116	(23, 53, 54)
Tri-2-*p*-cymyl-	87	(51)
Tri-*p*-cumyl-	159	(43)
Tri-*p*-dimethylaminophenyl-	230	(51)
Tri-*p*-fluorophenyl-	94	(51)
Trimesityl-	137	(51, 121)
Tri-α-naphthyl-	235	(14, 53, 54, 108)
Tri-β-naphthyl-	84	(108)
Tri-*p*-nitrophenyl-	121	(109, 111)
Trinitrophenyl-	159	(118)
Tri-2-nitro-*p*-tolyl-	126	(111)
Tri-*o*-phenetyl-	122	(54)
Tri-*p*-phenetyl-	73	(43, 54)
Tri-β-phenylethyl-		(55)
Triphenyl-	77.6	(14, 18, 29, 67, 83, 84, 97, 100, 108, 121)
Tristyryl-		(37)
Trithymyl-		(37)
Tri-α-thienyl-	137.5	(72)
Tri-*o*-tolyl-	129	(43, 53, 108, 110)
Tri-*m*-tolyl-	65	(18)
Tri-*p*-tolyl-	120	(15, 48, 53, 54, 83, 108, 109)
Tri-*m*-xylyl-	175	(83, 108, 110)
Tri-*p*-xylyl-	194.5	(43, 108)
Diphenyl-α-naphthyl-	119	(13, 14, 18, 23, 51, 52)
Di-*p*-tolyl-α-naphthyl-	130	(51, 52)
Di-*p*-chlorophenyl-α-naphthyl-	139	(51, 52)
Di-*p*-anisyl-α-naphthyl-	136	(51)
Di-*p*-phenetyl-α-naphthyl-	132	(51)
Dimesityl-α-naphthyl-	151.5	(51)
Di-*p*-chlorophenyl-*o*-tolyl-	104.5	(51)
Di-*p*-tolyl-*p*-chlorophenyl-	97	(51)
Di-*o*-tolyl-α-naphthyl-	114	(51)
Diphenyl-*p*-chlorophenyl-	83.5	(51)
Diphenyl-*p*-hydroxyphenyl-	180	(52)

TABLE 1—*Concluded*

ALIPHATIC COMPOUNDS	BOILING POINT	REFERENCES
	°C.	
Trimethyl-	110 at 760 mm.	(66, 75, 80, 81, 87, 92, 93)
Triethyl-	96 at 50 mm. 107 at 79 mm. 123 at 150 mm.	(8, 31, 34, 36, 47, 76, 80, 81, 93)
Tri-*n*-propyl-	87 at 8 mm. 127 at 50 mm.	(31)
Tri-*n*-butyl-	124 at 7 mm. 173 at 50 mm.	(31, 54)
Tri-*tert*-butyl-	Decomposes	(55)
Triisobutyl-	162 at 74 mm.	(15, 81)
Tri-*n*-amyl-	158 at 7 mm. 209 at 50 mm.	(31)
Triisoamyl-	200 at 70 mm.	(81)
Tricyclohexyl-		(16, 62, 112)
Diethylamyl-		(90)
Ethylcyclopentamethylene-	118 at 20 mm.	(63)
Trilauryl-		(107)
Tricetyl-		(107)

* Where more than one value for a melting point is given, the highest value is recorded in this and other tables.

decompose into the metal and biaryl compounds. Cambi (10) found that triphenylbismuth and diphenylmercury form no bimolecular compound. Challenger and Ridgway (23) have reported the interchange of aromatic radicals between bismuth and mercury compounds at high temperatures. Tri-α-naphthylbismuth and diphenylmercury at 200°C. gave di-α-naphthylmercury, triphenylbismuth, and diphenyl-α-naphthylbismuth. Tri-*p*-tolylbismuth and diphenylmercury at 180°C. gave triphenylbismuth and di-*p*-tolylmercury. No exchange occurred between tri-*p*-tolylbismuth and di-α-naphthylmercury at this temperature.

Calingaert and Beatty (11b) have described similar reactions as the "random intermolecular exchange of organic radicals." Thus, a mixture of tetraethyllead and tetramethyltin, after undergoing redistribution, yielded all ten possible R_4M compounds. Later, Calingaert, Soroos, and Thomson (11c) showed that tetramethyllead and diethylmercury, and tetraethyllead and dimethylmercury, both underwent a similar reaction to yield all the possible redistribution products.

There appears to be an exchange of radicals when two bismuth compounds are fused, since tri-α-naphthylbismuth and triphenylbismuth at 190°C. yield diphenyl-α-naphthylbismuth. No reaction occurred between tri-*p*-tolylbismuth and tri-α-naphthylbismuth at 200°C. There is evidence that the former reaction is reversible, and Calingaert, Soroos, and Hnizda (11a) have proposed this reaction as an example of random distribution (51).

With strong inorganic acids triarylbismuth compounds are cleaved quantitatively to the hydrocarbon and the inorganic bismuth salt (84). With the more weakly acidic compounds it is possible to isolate intermediate products (25, 55).

$$(C_6H_5)_3Bi + \left\{\begin{array}{lll} HSCN & \rightarrow (C_6H_5)_2BiSCN & + C_6H_6 \\ 2HSCN & \rightarrow C_6H_5Bi(SCN)_2 & + 2C_6H_6 \\ 2HSC_6H_5 & \rightarrow C_6H_5Bi(SC_6H_5)_2 & + 2C_6H_6 \end{array}\right.$$

Triphenylantimony is not cleaved by thiophenol under similar conditions (55). Tri-α-naphthylbismuth is not cleaved by this reagent under conditions which give 43.6 per cent cleavage with triphenylbismuth. Cleavage reactions with the halogens and pseudohalogens also indicate that the α-naphthyl radical is held more firmly to bismuth than is the phenyl radical (17, 18).

Table 1 lists all the known tertiary organobismuth compounds.

3. Unsymmetrical triarylbismuth compounds

Prior to a recent publication (51) the only unsymmetrical organobismuth compound which had been described in any detail was diphenyl-α-naphthylbismuth, prepared by Challenger and coworkers (13, 14, 15, 16, 17, 18, 19, 23).

$$(C_6H_5)_2BiBr + \alpha\text{-}C_{10}H_7MgBr \rightarrow (C_6H_5)_2BiC_{10}H_7\text{-}\alpha + MgBr_2 \tag{a}$$

Incidental to studies concerned with optically active organobismuth compounds, these authors tried unsuccessfully to prepare other unsymmetrical organobismuth compounds. More recently, Norvick (90) reported the formation of diethylamylbismuth, together with triethylbismuth and triamylbismuth, from the reaction between amylbismuth dichloride and ethylmagnesium bromide, but no physical data on this compound were provided.

On the basis of general observations concerned with the relative reactivities of organometallic compounds, it appeared quite probable that a variety of unsymmetrical compounds could be made and would be found to be reasonably stable. This was found to be the case. The procedures used are illustrated in the following reactions for the synthesis of di-*p*-tolyl-α-naphthylbismuth.

$$(p\text{-}CH_3C_6H_4)_2BiX + \alpha\text{-}C_{10}H_7MgBr \rightarrow (p\text{-}CH_3C_6H_4)_2BiC_{10}H_7\text{-}\alpha + MgXBr \tag{b}$$

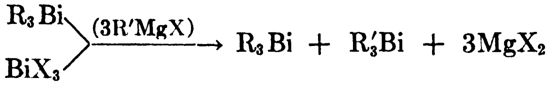
$$2p\text{-}CH_3C_6H_4MgBr + \alpha\text{-}C_{10}H_7BiBr_2 \rightarrow (p\text{-}CH_3C_6H_4)_2BiC_{10}H_7\text{-}\alpha + 2MgBr_2 \tag{c}$$

$$(p\text{-}CH_3C_6H_4)_2BiNa + \alpha\text{-}C_{10}H_7I \rightarrow (p\text{-}CH_3C_6H_4)_2BiC_{10}H_7\text{-}\alpha + NaI \tag{d}$$

Reaction (d) was carried out in liquid ammonia (52). Diarylbismuth halides react in liquid ammonia with sodium, potassium, lithium, calcium, and barium to form highly reactive R_2BiM or $(R_2Bi)_2M$ compounds which upon treatment with α-iodonaphthalene yield the corresponding unsymmetrical compound.

Once obtained in a pure condition, the unsymmetrical compounds were found to have a distinctly higher stability than might have been expected. For example, a sample of diphenyl-α-naphthylbismuth was kept in a stoppered bottle for three years without alteration in melting point. In this connection it is interesting to recall the observations of Calingaert, Soroos, and Hnizda (11a) that the reputedly unstable, unsymmetrical methylethylmercury was in reality quite stable and could be distilled without undergoing disproportionation, provided that traces of RHgX or other related catalysts are absent.

The general problem of redistribution or disproportionation of radicals is closely related to other reactions leading to the synthesis of the unsymmetrical organobismuth compounds. Challenger and coworkers found that, with the single exception of reaction (a), R_2BiX and $R'MgX$ compounds always yielded R_3Bi compounds. This indicated to them the possibility that an R_2BiX compound was a double salt of R_3Bi and BiX_3. The use of a sufficient excess of $R'MgX$ should have yielded two symmetrical organobismuth compounds.



$$\left.\begin{array}{l} R_3Bi \\ BiX_3 \end{array}\right\rangle \xrightarrow{(3R'MgX)} R_3Bi + R'_3Bi + 3MgX_2$$

Although this did not occur when R and R′ were aryl radicals (since only R_3Bi was isolated), when R′ was an alkyl radical the odor of an alkylbismuth compound was evident. It is our opinion that, although organometallic salts in general show the equilibria,

$$R_2MX \rightleftarrows RMX_2 \rightleftarrows R_3M + MX_3$$

some of the unsymmetrical $R_2R'Bi$ compound was formed initially and then may have undergone redistribution, in part or in whole, to R_3Bi and R'_3Bi, to account for the formation of a trialkylbismuth compound.

4. The preferential cleavage of radicals from bismuth in unsymmetrical triarylbismuth compounds

Challenger and Allpress (17) first demonstrated the preferential cleavage of radicals in unsymmetrical organobismuth compounds when they reported that diphenyl-α-naphthylbismuth and iodine bromide yielded α-iodonaphthalene and diphenylbismuth bromide.

$$(C_6H_5)_2BiC_{10}H_7\text{-}\alpha + IBr \rightarrow (C_6H_5)_2BiBr + \alpha\text{-}C_{10}H_7I$$

It was found possible to extend the metal–metal interconversion reaction between organobismuth compounds and *n*-butyllithium (54),

$$(p\text{-}CH_3C_6H_4)_3Bi + 3n\text{-}C_4H_9Li \rightarrow 3p\text{-}CH_3C_6H_4Li + (n\text{-}C_4H_9)_3Bi$$

to determine the relative labilities of radicals attached to bismuth. When an unsymmetrical organobismuth compound, $R_2R'Bi$, was treated with one equivalent of *n*-butyllithium, there was a preferential replacement of one of two available radicals (55). In most instances both R and R′ were cleaved; for example, when diphenyl-α-naphthylbismuth and one equivalent of *n*-butyllithium were allowed to react for 10 min., in diethyl ether, there was obtained, subsequent to carbonation, yields of 21.6 per cent of α-naphthoic acid and 1.7 per cent of benzoic acid. This single experiment had a particular significance, since it might have appeared that the ratio of 2R:R′ in an $R_2R'Bi$ compound was unfavorable to R′ in the determination of the preferential cleavage of either R or R′. If the relative amounts of the two radicals were the sole consideration, the law of probability would have indicated that more R groups would cleave than would R′. This was not found to be true, however, with the above compound or with a number of other $R_2R'Bi$ compounds. Furthermore, while cleavage of diphenyl-α-naphthylbismuth by *n*-butyllithium showed the α-naphthyl and phenyl radicals to possess labilities which might have been predicted on the basis of the series of radicals obtained by the cleavage of unsymmetrical RR′Hg compounds by hydrogen chloride (69b), this was the exception. Anomalously, the *p*-chlorophenyl radical was cleaved as readily as the α-naphthyl, while the phenyl radical was cleaved in preference to the *p*-tolyl and *o*-tolyl groups.

Similar reactions between symmetrical R_3Bi compounds and *n*-butyllithium gave confirmation to the data obtained with the unsymmetrical bismuth compounds, and, in addition, provided further exceptions to the series of radicals mentioned above (69b).

The series of radicals obtained by this metal–metal interconversion reaction, in order of decreasing ease of cleavage from bismuth, was as follows: (*p*-chlorophenyl, *p*-bromophenyl, *p*-fluorophenyl, α-naphthyl), phenyl, *p*-tolyl, *p*-ethoxyphenyl, (*o*-tolyl, mesityl, *o*-chlorophenyl).

On the basis of cleavage from unsymmetrical mercurials by hydrogen chloride, these radicals possess the following order of decreasing lability: mesityl, α-naphthyl, *o*-tolyl, *p*-tolyl, *p*-fluorophenyl, phenyl, (*p*-chlorophenyl, *p*-bromophenyl), *o*-chlorophenyl.

The *p*-ethoxyphenyl radical is not listed in this latter series, but the *p*-methoxyphenyl radical is placed above the *p*-tolyl radical and approximately equal to the mesityl radical in lability. One may arbitrarily assume that the *p*-methoxyphenyl group would occupy a position similar to the *p*-ethoxyphenyl radical.

It is interesting to note that dimesitylmercury was cleaved to the extent of 72.5 per cent under the same conditions which gave no cleavage with trimesitylbismuth.

The solvent played an important rôle in all of these reactions. Cleavage of the *p*-chlorophenyl radical proceeded to the extent of 50 per cent in diethyl ether, but no cleavage occurred in a mixture of petroleum ether and benzene. The effect of the solvent manifested itself, also, in the low order of reaction between tri-α-naphthylbismuth and *n*-butyllithium, where the yield of α-naphthoic acid was 1.2 per cent. In all the other reactions described above, the bismuth compounds were completely dissolved in the solvent prior to the addition of the *n*-butyllithium. Tri-α-naphthylbismuth is practically insoluble in diethyl ether and the slow rate of reaction was due probably to this. With the ether-soluble unsymmetrical bismuth compounds containing the α-naphthyl groups, the radical showed itself to have a lability equal to that of the *p*-chlorophenyl radical. The use of a benzene–ether mixture, in which tri-α-naphthylbismuth is soluble, decreased the yield of α-naphthoic acid to 0.6 per cent.

5. *Compounds of the general formulas* R_2BiX *and* $RBiX_2$

(a) Alkyl compounds

Alkyl bismuth compounds of the type R_2BiX (where X is Cl or Br) are crystalline solids which burn spontaneously in air and must, therefore, be isolated under an inert atmosphere. They are soluble in alcohol and insoluble in ether. The following methods have been employed in their preparation:

(*a*) *Reaction between a trialkylbismuth compound and chlorine or bromine:* The cooled ether solution of the trialkylbismuth compound is treated with the calculated quantity of the halogen (80, 81).

(*b*) *Reaction between tetraethyllead and bismuth chloride:* The reaction between tetraethyllead and bismuth chloride yields a colorless solid which ignites spontaneously in air and appears to consist in part of diethylbismuth chloride (50).

Compounds of the general formula $RBiX_2$ are more stable than the R_2BiX type discussed above. These, too, are solids possessing high melting points and are stable in air. They are somewhat soluble in alcohol and almost insoluble in ether and petroleum ether. The following methods have been employed in their preparation:

(*a*) *Reaction between a bismuth halide and a tertiary bismuth compound:* To a solution of bismuth chloride or bromide is added the calculated quantity of the R_3Bi compound. The $RBiX_2$ compound separates out as a yellow powder (80, 81).

(*b*) *Reaction between a Grignard reagent and a bismuth halide:* Methylbismuth dibromide has been prepared in small yields by the reaction between methylmagnesium iodide and bismuth bromide (114). Dimethylzinc and bismuth bromide also yield methylbismuth dibromide (80). Ethylbismuth dibromide has been obtained by the interaction of bismuth bromide and ethylmagnesium bromide (63).

(*c*) *Reaction between an* $RBiCl_2$ *compound and potassium iodide:* Dünhaupt (34) prepared ethylbismuth diiodide by treating the corresponding dichloride with potassium iodide.

(*d*) *Reaction between a tertiary bismuth compound and an alkyl iodide:* Derivatives of the type $RBiI_2$ are produced when a tertiary bismuth compound and an alkyl iodide are heated to about 200°C.

(*e*) *Reaction between a tertiary bismuth compound and an inorganic halide:* Inorganic halides, e. g., silver nitrate or mercuric chloride, readily cleave bismuth compounds and are themselves reduced to the free metal.

When an alkylbismuth dihalide is treated with aqueous ammonia or sodium hydroxide, an oxide, RBiO, is formed. This oxide is easily oxidized further by air. With ethylbismuth diiodide, alcoholic silver nitrate forms ethylbismuth dinitrate. Marquardt (80) has reported that dimethylbismuth bromide forms a double salt with zinc bromide. This double salt when treated with alcoholic ammonia yields methylbismuth oxide; with methyl iodide it forms methylbismuth diiodide, and with water it forms dimethylbismuth hydroxide.

(b) Aryl compounds

Aromatic bismuth halides of the general formula R_2BiX or $RBiX_2$ are crystalline solids possessing high melting points. Generally speaking, compounds of these types are decomposed by moisture, alcohol, and ammonia. It was found, however, that *o*-carbomethoxyphenylbismuth dichloride, di-*o*-carbomethoxyphenylbismuth chloride, and di-*o*-carboethoxyphenylbismuth chloride were unaffected by moisture or alcohol and could be kept indefinitely without any precautions being taken (53).

Extensive studies of compounds like $(C_6H_5)_3BiX_2$ have shown that the order of decreasing stability of the dihalides is R_3BiCl_2, R_3BiBr_2, R_3BiI_2. With R_2BiX compounds the order seems to be reversed, based on ammonolytic and hydrolytic reactions, as well as conversion to R_3Bi by hydrazine (49, 52).

Compounds of the general formula R_2BiX and $RBiX_2$ have been obtained in a variety of ways:

(*a*) *Reaction between a tertiary bismuth compound and a bismuth halide:* The calculated quantity of anhydrous bismuth chloride or bismuth bromide in dry ether is added to an ether solution of the tertiary bismuth compound. Two moles of bismuth compound and one of bismuth halide yield R_2BiX compounds.

$$2R_3Bi + BiX_3 \rightarrow 3R_2BiX$$

The yields are very good, in many instances almost quantitative (51). The diarylbismuth chlorides are insoluble in most organic solvents but can be recrystallized from large volumes of dry benzene. The diarylbismuth bromides are slightly soluble in ether. If the molar ratio of bismuth halide and bismuth compound is reversed, $RBiX_2$ compounds are formed.

$$R_3Bi + 2BiX_3 \rightarrow 3RBiX_2$$

Yields, here, are also good. The dichlorides are extremely insoluble, but the dibromides, with the exception of α-naphthylbismuth dibromide, are quite soluble in ether. These are the best methods available for the preparation of aromatic R_2BiX and $RBiX_2$ compounds.

(*b*) *Reaction between a tertiary bismuth compound and an inorganic halide other than bismuth chloride:* See page 293.

(*c*) *Reaction between tetraphenyllead and a bismuth halide:* Tetraphenyllead and bismuth bromide yield diphenylbismuth bromide and diphenyllead dibromide (58).

(*d*) *Reaction between an* R_2BiCl *compound and potassium iodide:* Diphenylbismuth iodide, di-*p*-chlorophenylbismuth iodide, and di-*p*-tolylbismuth iodide have been prepared by the interaction of the corresponding chloride and potassium iodide in absolute alcohol (7, 51).

(*e*) *Reaction between a tertiary compound and a halogen, halogen halide, or halogen pseudohalide:* See page 292.

(*f*) *Reaction between an* R_2BiX *compound and a halogen:* Challenger (14) prepared phenylbismuth dibromide by treating diphenylbismuth bromide with bromine in chloroform. Diphenylbismuth bromide and iodine monochloride

TABLE 2

R_2BiX *and* $RBiX_2$ *compounds*

COMPOUND	MELTING POINT	REFERENCES
	°C.	
Dibiphenylbismuth chloride		(120)
Di-*p*-chlorophenylbismuth chloride	160	(51)
Di-*p*-chlorophenylbismuth bromide	159	(23)
Di-*p*-chlorophenylbismuth iodide	140	(23, 51)
Di-*α*-naphthylbismuth chloride	168	(17, 18, 59)
Di-*α*-naphthylbismuth bromide (?)*		(17)
Di-*α*-naphthylbismuth iodide (?)		(17)
Diphenylbismuth chloride	185	(17, 18, 21, 23, 51)
Diphenylbismuth bromide	158	(14, 17, 51, 83)
Diphenylbismuth iodide	134	(7, 17, 43, 51)
Diphenylbismuth selenocyanate	Decomposes	(20)
Diphenylbismuth azide	168	(22)
Diphenylbismuth cyanide	210	(17, 20, 26)
Diphenylbismuth hydroxide		(83, 100)
Diphenylbismuth thiocyanate	123.5	(25, 26)
Di-*p*-tolylbismuth chloride	181.5	(15, 18, 51, 59)
Di-*p*-tolylbismuth selenocyanate		(20)
Di-*p*-tolylbismuth iodide	148	(51)
Di-*o*-carboethoxyphenylbismuth chloride	148	(53)
Di-*o*-carbomethoxyphenylbismuth chloride	181	(53)
Phenylbismuth chlorobromide		(16, 17)
Phenylbismuth dibromide	206	(14, 18, 51)
Phenylbismuth dichloride (?)	74	(74)
Phenylbismuth diiodide	194	(17)
Phenylbismuth dithiocyanate (?)	186	(25)
p-Chlorophenylbismuth dibromide	244	(23)
α-Naphthylbismuth dibromide	208	(13, 18, 51)
p-Tolylbismuth dichloride	207	(18)
o-Tolylbismuth dibromide	181	(51)
Phenylbismuth oxide (?)	233	(74)
o-Carbomethoxyphenylbismuth dichloride	221	(53)
o-Methoxyphenylbismuth dibromide	171	(55)
Diethylbismuth bromide		(80)
Diethylbismuth iodide (?)		(34)
Dimethylbismuth chloride	116	(80)
Dimethylbismuth bromide		(80)
Dimethylbismuth hydroxide		(80, 81)
Diisobutylbismuth bromide		(81)
Diisoamylbismuth bromide		(81)
Methylbismuth dichloride	242	(80)
Methylbismuth dibromide	214	(80, 114)
Methylbismuth diiodide		(80)
Methylbismuth oxide		(80, 81)
Methylbismuth sulfide (?)		(81)
Ethylbismuth dichloride		(34, 80)
Ethylbismuth sulfide (?)		(34)
Ethylbismuth dibromide		(34, 80)

TABLE 2—*Concluded*

COMPOUND	MELTING POINT	REFERENCES
	°C.	
Ethylbismuth oxide (?)		(34)
Ethylbismuth diiodide		(34, 80)
Ethylbismuth carbonate (?)		(34)
Isobutylbismuth dibromide	124	(81)
Isobutylbismuth diiodide		(81)
Isoamylbismuth dibromide	134	(81)
Amylbismuth dichloride		(90)
Cetylbismuth dibromide		(107)

* A question mark following any compound indicates that its composition as indicated is open to some criticism, owing either to the fact that the compound was not analyzed, or to the fact that the analyses given by the workers were not conclusive.

yielded phenylbismuth chlorobromide (16). Diphenylbismuth iodide and bromine gave phenylbismuth dibromide (16).

(*g*) *Cleavage of a tertiary bismuth compound during the preparation of the dichloride:* Challenger and Wilkinson (26) have obtained small quantities of diphenylbismuth chloride during the preparation of triphenylbismuth dichloride from triphenylbismuth and chlorine. Considerable quantities of this compound are formed if the chloroform solution of the tertiary compound is not cooled in ice prior to the introduction of the chlorine (55).

Table 2 lists all the known R_2BiX and $RBiX_2$ compounds.

6. Pentavalent bismuth compounds

The pentavalent bismuth derivatives are the most stable of the organobismuth compounds.[4] They are crystalline solids which are soluble in chloroform, dioxane, benzene, and acetone; they are insoluble in alcohol and ether.

Triarylbismuth dichlorides may be prepared by passing a stream of dry gaseous chlorine through the ice-cold solution of the tertiary compound in chloroform until an excess of the gas is present. The chloroform is then partially evaporated, and the addition of several volumes of methanol precipitates the dichloride in a high state of purity. The yields are generally better than 90 per cent. An alternative method is to pass a stream of dry chlorine over a petroleum ether solution of the tertiary compound. This method has the disadvantage that triarylbismuth compounds are not very soluble in this solvent and it is necessary to work with large volumes of solution. Its advantage, however, is that the dichloride which forms is insoluble in petroleum ether and separates out immediately. Thus, nuclear chlorination is not possible. Trimesitylbismuth forms a dichloride readily in this solvent (121), but if the preparation is carried out in benzene or chloroform nuclear or lateral substitution occurs (51). Sulfuryl chloride, sulfur monochloride, thionyl chloride (15, 18), and iodine trichloride

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[4] The only exceptions are the action of alkali and heat, which decompose R_3BiX_2 compounds more rapidly than R_3Bi compounds.

(19) also yield dichlorides when reacted with tertiary bismuth compounds, but some cleavage occurs simultaneously.

Triphenylbismuth difluoride is prepared by the interaction of triphenylbismuth dichloride and potassium fluoride.

Triarylbismuth dibromides are prepared by the dropwise addition of a solution of bromine in carbon tetrachloride to an ice-cold solution of the tertiary compound in chloroform or petroleum ether. The first slight excess of bromine is easily visible and can be used to indicate the end point of the reaction. From chloroform the dibromide is isolated by partial evaporation of the solvents and precipitation with methanol. The dibromide separates out of the petroleum ether directly. Yields are generally about 90 per cent.

The halogens in R_3BiX_2 compounds are quite labile and undergo double decomposition reactions readily.

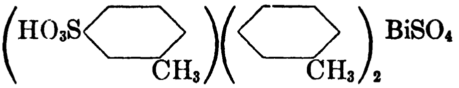
$$(C_6H_5)_3BiX_2 + \begin{cases} 2AgOCN \rightarrow (C_6H_5)_3Bi(OCN)_2 + 2AgX \\ 2C_6H_5COOAg \rightarrow (C_6H_5)_3Bi(OOCC_6H_5)_2 + 2AgX \\ 2\text{-}o\text{-}HOC_6H_4COONa \rightarrow (C_6H_5)_3Bi(OOCC_6H_4OH\text{-}o)_2 \\ \qquad\qquad\qquad\qquad\qquad + 2NaX \\ (CH_3COO)_2Pb \rightarrow (C_6H_5)_3Bi(OOCCH_3)_2 + PbX_2 \\ 2AgOH \rightarrow (C_6H_5)_3Bi(OH)_2 + 2AgX \end{cases}$$

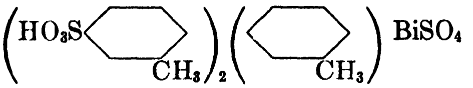
Supniewski and Adams (111) have reported that the side chains of tri-*o*- and tri-*p*-tolylbismuth dichlorides are oxidized by potassium permanganate, lead tetraacetate, or chromic acid. Yields range from 15 to 25 per cent.

$$(CH_3C_6H_4)_3BiCl_2 \rightarrow (HO_2CC_6H_4)_3BiCl_2$$

The compounds are soluble in alkali but upon reprecipitation by hydrochloric acid yield low-melting solids. Alkaline solutions of these compounds appear to be unstable and deposit a yellow solid on standing. The tri-1,3-xylylbismuth dichloride and the tri-*m*-tolylbismuth dichloride were oxidized also, but the products were not isolated. The permanganate oxidation appears difficult to repeat (55).

Supniewski (110) was the first to report the successful nuclear sulfonation of tri-*o*-tolylbismuth dichloride. He obtained two compounds, 4-sulfo-2-methylphenyldi-*o*-tolylbismuth sulfate and a corresponding di-4-sulfo-2-methylphenyl-*o*-tolylbismuth sulfate.





These compounds were soluble in alkali and were precipitated by acid. Once precipitated by acids, however, they did not redissolve readily in alkali.

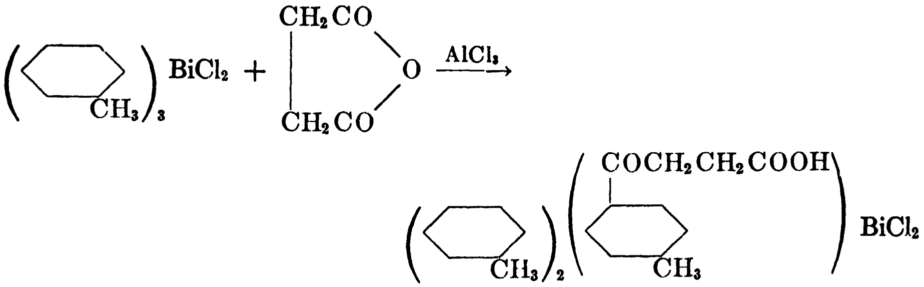
The nuclear nitration of aromatic bismuth compounds is carried out successfully only at comparatively low temperatures and with the triarylbismuth dinitrates. In order to form the corresponding trinitro derivative, triphenylbismuth dinitrate was nitrated at 0°C. for 12 hr. with fuming nitric acid, and tri-*p*-tolylbismuth dinitrate for 2 hr. at room temperature (111). Wilkinson and Challenger (118) have prepared a hexanitro derivative by nitration at −10°C. for 68 hr.

The orienting influence of bismuth in triarylbismuth compounds is not readily determined, since the compounds are decomposed easily by fuming nitric acid. A neutral nitrating agent, benzoyl nitrate, and triphenylbismuth yielded only triphenylbismuth dinitrate (118).

The nitration of triphenylbismuth dinitrate yields, on the average, 86 per cent of the meta, 12 per cent of the ortho, and 2 per cent of the para isomers (24, 115). Supniewski and Adams (111) and Supniewski (109, 110) have reported several nitro derivatives, but they have not presented any structural proof for their compounds.

Triarylbismuth dihydroxides are prepared by treating the dichloride or dibromide with moist ammonia or silver hydroxide. The reaction proceeds through two steps, so that it is possible to isolate the hydroxyhalide. The dihydroxide will oxidize ethyl alcohol to acetaldehyde, isopropyl alcohol to acetone, and *n*-propyl alcohol to propionaldehyde (22). With carbon dioxide the dihydroxide forms a carbonate, and with hydrochloric acid it forms a dichloride.

The attempted preparation of water-soluble organobismuth compounds by the reaction between maleic or succinic anhydride and a triarylbismuth dichloride has been generally unsuccessful (55).



If mild conditions were employed, the triarylbismuth dichloride was recovered unreacted. When more drastic conditions were employed, the bismuth compound was partially or wholly destroyed without any indication of the desired reaction having occurred.

Supniewski and Adams (111) attempted to cleave the ether linkage in tri-*p*-anisylbismuth dibromide with hydriodic acid and with aluminum chloride. These reactions resulted only in the complete decomposition or the recovery of unchanged bismuth compound. Similarly, an attempted lateral bromination, in the presence of ultraviolet light, of tri-*o*-tolylbismuth dibromide and tri-*p*-tolylbismuth dibromide resulted only in the cleavage of the carbon–bismuth linkage. When triphenylbismuth dichloride, ethyl chloroacetate, and copper bronze were refluxed in chloroform, only triphenylbismuth was obtained. There was no evidence that the pentavalent compound, $(C_6H_5)_3Bi(CH_2COOC_2H_5)_2$ had formed (55).

Triphenylbismuth hydroxychloride when treated with hydrobromic acid did not give triphenylbismuth chlorobromide. The products isolated were equimolecular quantities of triphenylbismuth dichloride and dibromide (17). No compounds of the type R_3BiXX' have been prepared, although phenylbismuth chlorobromide can be obtained in 77 per cent yield.

An unusual application of triarylbismuth dihalides has been their use in the color test described recently (56). The test is based on the observation made some years ago by Challenger and coworkers (14, 19, 26) that a transitory purple color developed when triphenylbismuth dibromide reacted with phenylmagnesium bromide incidental to an attempted preparation of tetraphenylbismuthonium bromide. The reaction apparently did not proceed as expected,

$$(C_6H_5)_3BiBr_2 + C_6H_5MgBr \rightarrow (C_6H_5)_4BiBr + MgBr_2$$

since the only products obtained were triphenylbismuth, diphenylbismuth bromide, phenylbismuth dibromide, and bromobenzene.

Two suggestions were offered to account for the transient purple color. One of these was the possible formation of tetraarylbismuthonium halides, and the other was the momentary formation of compounds of the type R_5Bi, analogous to the bright red triphenylmethyltetramethylammonium and benzyltetramethylammonium prepared by Schlenk and Holtz (103, 104). These are the only R_5 types reported in Group V. The purple color is probably not due to the formation of a bismuthonium halide, because R_4AsX and R_4SbX compounds are colorless. The explanation based on the formation of an R_5Bi compound is somewhat vitiated by the fact that triphenylantimony dichloride, tri-*p*-tolylantimony dichloride, tributylantimony dibromide, or triphenylarsenic dichloride and an arylmagnesium halide do not give a purple color. Although organoantimony and organobismuth compounds have some decidedly different chemical properties, it might be expected that an R_5Sb or R_5As compound, if formed, should also give a purple color.

The color test is carried out by adding 1 ml. of the RM solution to 1 ml. of an approximately 1 per cent solution of triphenylbismuth dichloride in dry benzene. With *aryl*lithium and *aryl*magnesium compounds a deep purple color forms instantaneously. To detect the presence of other aromatic RM compounds, which do not give this purple color, the test solution, obtained as described above, is heated to boiling, cooled, and hydrolyzed with 1 ml. of water. The benzene layer is then colored yellow, yellow-brown, or yellow-orange when the test is positive. These hydrolysis colors are obtained also by hydrolyzing the purple solutions obtained with RLi and RMgX compounds. No color is obtained before or after hydrolysis with *alkyl*metallic compounds like ethylmagnesium bromide, benzyllithium, or phenylethynyllithium.

A large number of triarylbismuth dihalides give the test. This applies to both symmetrical (R_3BiX_2) and unsymmetrical ($R_2R'BiX_2$) types. Steric

TABLE 3

Pentavalent bismuth compounds

COMPOUND	MELTING POINT	REFERENCES
	°C.	
Triarylbismuth difluorides:		
Triphenyl-	159	(26)
Triarylbismuth dichlorides:		
Tri-*o*-anisyl-	174	(108, 110)
Tri-*p*-anisyl-	133	(110)
Trichloro-*p*-anisyl-	133	(43)
Tribiphenyl-	200 d.*	(120)
Tri-*p*-bromophenyl-	155	(23)
Tri-*p*-chlorophenyl-	170	(23, 55)
Tri-*p*-cumyl-	208	(43)
Tri-2-*p*-cymyl-	164	(51)
Trimesityl-	150	(121)
Dichlorotrimesityl-	150	(51)
Tri-*α*-naphthyl-	174	(18, 19, 55)
Triphenyl-	141.5	(15, 19, 26, 83, 84, 109, 121)
Tri-*o*-carboxyphenyl-	159	(110, 111)
Tri-*o*-carbomethoxyphenyl-	137	(110)
Tri-*p*-carboxyphenyl-	130	(109, 111)
Tri-*p*-carbomethoxyphenyl-	107	(110, 111)
Trinitrophenyl-	128	(118)
Tri-*p*-nitrophenyl	134	(109, 111)
Dinitrotriphenyl-	136	(43, 118)
Tetranitrotriphenyl-	146	(118)
Mononitrotriphenyl-	110	(118)
Hexanitrotriphenyl-	148	(118)
Tri-*p*-carbomethoxy-*o*-nitrophenyl-	260 d.	(110, 111)
Tri-*α*-thienyl-		(73)
Tri-*o*-tolyl-	162	(43, 108, 110)
Tri-*m*-tolyl-	133	(111)
Tri-*p*-tolyl-	147	(18, 83, 108, 109)
Tri-3-nitro-*o*-tolyl-	150	(110, 111)
Tri-2-nitro-*p*-tolyl-	156	(109, 111)
Tri-*m*-xylyl-	161	(83, 110)
Tri-*p*-xylyl-	167.5	(43)
Diphenyl-*α*-naphthyl-	142	(26)
Di-*p*-tolyl-*α*-naphthyl-	147	(51)
Di-*p*-chlorophenyl-*α*-naphthyl-	132	(51)
Di-*p*-chlorophenyl-*o*-tolyl-	133	(51)
Di-*o*-tolyl-*α*-naphthyl-	140	(51)
Diphenyl-*p*-tolyl-	110	(51)
Triarylbismuth dibromides:		
Tri-*o*-anisyl-	101	(108)
Tri-*p*-anisyl-	103	(43, 111)
Tribiphenyl-	Decomposes	(120)
Tri-*p*-bromophenyl-	107 d.	(23)
Tri-*p*-chlorophenyl-	105	(23)
Tri-*p*-cumyl-	150	(43)
Tri-2-*p*-cymyl-	103	(51)
Trimesityl-	Above 250	(121)
	93 d.	(51)

TABLE 3—*Concluded*

COMPOUND	MELTING POINT	REFERENCES
Triarylbismuth dibromides—*continued:*	°C.	
Tri-α-naphthyl-	124	(14, 108)
Tri-*o*-phenetyl-	128	(51)
Triphenyl-	124	(14, 83, 84)
Tri-*o*-tolyl-	127	(43, 108)
Tri-*m*-tolyl-	92	(21, 111)
Tri-*p*-tolyl-	114	(83, 108, 109)
Tri-*m*-xylyl-	117	(83)
Tri-*p*-xylyl-	130	(43)
Diphenyl-α-naphthyl-	140	(19, 26)
Di-*p*-tolyl-α-naphthyl	127	(51)
Di-*p*-chlorophenyl-α-naphthyl-	103	(51)
Di-*p*-chlorophenyl-*o*-tolyl-	110	(51)
Di-*o*-tolyl-α-naphthyl-	122	(51)
Triarylbismuth diiodides:		
Triphenyl-	Stable at −78	(118)
Triarylbismuth hydroxychlorides:		
Triphenyl-	161	(19, 22)
Tri-*p*-tolyl-	154 d.*	(108)
Trihydroxy-*o*-tolyl-	154	(111)
Triarylbismuth hydroxybromides:		
Triphenyl-	148	(19)
Tri-*p*-tolyl-	140	(108)
Tri-*m*-xylyl-	250 d.	(108)
Triarylbismuth dinitrates:		
Tribiphenyl-	162 d.	(120)
Triphenyl-	130 d.	(83, 109, 115, 118)
Tri-*p*-carbomethoxy-*x*-nitrophenyl-	160 d.	(111)
Tri-*m*-nitrophenyl-	145 d.	(24, 115)
Tri-*p*-nitrophenyl-	147 d.	(109, 111)
Dinitrotriphenyl-	150 d.	(43)
Tetranitrotriphenyl-		(118)
Mononitrotriphenyl-		(118)
Hexanitrotriphenyl-		(118)
Tri-*o*-tolyl-		(43)
Tri-*p*-tolyl-		(83, 109)
Tri-2-nitro-*p*-tolyl-	160 d.	(109, 111)
Miscellaneous pentavalent derivatives:		
Triphenylbismuth carbonate	Above 220	(19, 83)
Triphenylbismuth diazide	93	(22)
Triphenylbismuth dicyanate	129	(27)
Triphenylbismuth hydroxycyanide	135	(22)
Triphenylbismuth oxide	120 d.	(15, 22, 83)
Tri-*o*-tolylbismuth oxide	100 d.	(55)
Tri-*p*-tolylbismuth oxide	109 d.	(55)
Triphenylbismuth sulfate	Above 284	(15, 19)
4-Sulfo-2-methylphenyldi-*o*-tolylbismuth sulfate	167	(110)
Tetranitrotriphenylbismuth oxide	108 d.	(118)
Di-4-sulfo-2-methylphenyl-*o*-tolvlbismuth sulfate	174	(110)

* d. = decomposes.

factors either in the RM compound or in the R_3BiX_2 compound decrease the intensity of the purple color, or may in some instances prevent its formation. For example, triphenylbismuth dichloride or tri-*p*-tolylbismuth dichloride (or dibromide) and mesitylmagnesium bromide gave no purple color, whereas with *o*-tolylmagnesium bromide a very intense purple color was obtained. Tri-*o*-tolylbismuth dichloride gave a weak purple color with phenylmagnesium bromide and with *p*-tolylmagnesium bromide, but a negative test with α-naphthylmagnesium bromide. Trimesitylbismuth dichloride gave a negative test with these three Grignard reagents. Challenger and Allpress (18) have reported no purple color in the reaction between tri-α-naphthylbismuth dibromide and phenylmagnesium bromide.

Diarylbismuth halides (R_2BiX) and arylbismuth dihalides ($RBiX_2$) do not give the color test.

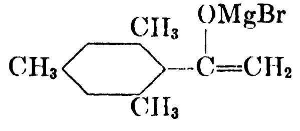
The color test is extremely sensitive. Five-hundredths of a milliliter of a 1.08 molar solution of *o*-tolylmagnesium bromide gave a positive test with 1 ml. of a 0.0078 molar solution of triphenylbismuth dichloride. This represents 0.004 g. of triphenylbismuth dichloride or 0.0016 g. of bismuth.

The color test has found application in studies on the rates and mechanisms of some metalation and halogen-metal interconversion reactions.

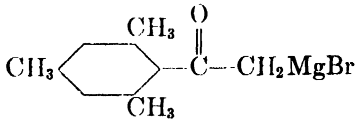
$$\text{Aryl—H} + n\text{-}C_4H_9Li \rightarrow \text{Aryl—Li} + n\text{-}C_4H_{10}$$

$$\alpha\text{-}C_{10}H_7Br + n\text{-}C_4H_9Li \rightarrow \alpha\text{-}C_{10}H_7Li + C_4H_9Br$$

A further application has to do with the bromomagnesium derivatives of sterically hindered ketones, like acetomesitylene, the enolates (A) of which behave like true organomagnesium compounds (B).



(A)



(B)

The bromomagnesium derivative of acetomesitylene, prepared from phenylmagnesium bromide and an excess of acetomesitylene, gave a positive color test with Michler's ketone (45b, 45c). Any uncertainty concerning the influence of phenylmagnesium bromide was ruled out, because the negative color test with triphenylbismuth dichloride showed that no *aryl*magnesium halide was present.

Table 3 lists all the known pentavalent organobismuth compounds.

7. *Reactions in liquid ammonia*

Diarylbismuth halides react smoothly with metals which dissolve in liquid ammonia to give deep red R_2BiM or $(R_2Bi)_2M$ compounds. The metals employed were lithium, sodium, potassium, calcium, and barium (52). The R_2BiM and $(R_2Bi)_2M$ compounds decompose slowly to the corresponding R_3Bi compound and inorganic bismuth, but they are sufficiently stable to react promptly with RX compounds. This provides an additional avenue of approach to the preparation of unsymmetrical organobismuth compounds.

$$R_2BiX + 2M \rightarrow R_2BiM + MX$$

$$R_2BiM + R'X \rightarrow R_2R'Bi + MX$$

The reactivity of the R_2BiM compounds is due in part to their solubility in liquid ammonia, for the insoluble sodium–bismuth alloy does not react with iodobenzene. Thus far it has not been possible to prepare these compounds directly from the R_3Bi compound and the metal.

$$R_3Bi + 2M \rightarrow R_2BiM + [RM]$$

The reaction between an arylbismuth dibromide and sodium does not lead to the expected $RBiNa_2$.

$$RBiBr_2 + 4Na \rightarrow RBiNa_2 + 2NaBr$$

Instead, a complex decomposition occurs and the only products are RH and inorganic bismuth.

Mention has been made (page 285) of the possible existence of the diarylbismuth radical in liquid ammonia.

A metal–halogen interconversion (46, 52) appears to take place in liquid ammonia between R_2BiM compounds and α-iodonaphthalene. In these reactions significant quantities of naphthalene are formed in addition to the diphenyl-α-naphthylbismuth. The naphthalene very probably does not come from the following reaction:

$$\alpha\text{-}C_{10}H_7I + 2Na \xrightarrow{NH_3} \alpha\text{-}C_{10}H_7Na \xrightarrow{NH_3} C_{10}H_8$$

since there is no free sodium in the R_2BiNa solutions. It appears, therefore, that the naphthalene may owe its formation to a new type of halogen–metal interconversion.

$$R_2BiNa + \alpha\text{-}C_{10}H_7I \rightarrow R_2BiI + \alpha\text{-}C_{10}H_7Na$$

$$\alpha\text{-}C_{10}H_7Na \xrightarrow{NH_3} C_{10}H_8 + NaNH_2$$

The liquid ammonia reactions make possible the preparation of water-soluble organobismuth compounds.

$$R_2BiM + p\text{-}BrC_6H_4OH \rightarrow R_2BiC_6H_4OH\text{-}p + MBr$$

$$R_2BiM + o\text{-}IC_6H_4COONa \rightarrow R_2BiC_6H_4COONa\text{-}o + MI$$

The phenolic compound was rather unstable, as might have been predicted, because of the lability of a hydroxyphenyl radical attached to a metal. The carboxylic derivative could not be obtained pure, owing primarily to the relative inertness of halogens ortho and para to the functional group in benzoic acid and benzoates towards R_2BiM compounds in liquid ammonia. Such halogens are much less reactive in this medium than the halogens in a simple halogenated benzene or naphthalene. There was evidence, however, of the presence of a water-soluble bismuth compound.

8. Organobismuth salts of organic acids

(a) Trivalent compounds

In studying the cleavage of tertiary bismuth compounds by thiophenol (page 297), the reaction products were isolated in order to determine their composition and possible therapeutic properties. It was of importance to determine what effect a C_6H_5S— group instead of a C_6H_5— group would have on the toxicity of bismuth in an organometallic molecule (55).

Early observations with triphenylbismuth and thiophenol showed that the only product obtained was the secondary cleavage product, even though an equimolecular ratio of the two compounds was used.

$$(C_6H_5)_3Bi + 2C_6H_5SH \rightarrow C_6H_5Bi(SC_6H_5)_2 + 2C_6H_6$$

This would indicate either of two possibilities: (*1*) that $(C_6H_5)_2BiSC_6H_5$ is unstable, or (*2*) that the second phenyl group was cleaved with greater ease apparently than was the first. The latter presumption is in contradiction with the general rule that a second R group is cleaved with greater difficulty than the first. Furthermore, while Catlin (12) found that primary cleavage of various RM compounds by means of hydrogen chloride is aided by chloroform as the solvent, secondary cleavage of triphenylbismuth by thiophenol occurred preferentially in the absence of a solvent or in chloroform, benzene, or toluene.

Further work with tri-*p*-chlorophenylbismuth and tri-*p*-tolylbismuth indicated that here, too, the secondary cleavage was preferential, since the products obtained were *p*-$ClC_6H_4Bi(SC_6H_5)_2$ and *p*-$CH_3C_6H_4Bi(SC_6H_5)_2$, respectively. Triphenylbismuth and methyl thiosalicylate also gave the corresponding secondary cleavage product, $C_6H_5Bi(SC_6H_4COOCH_3\text{-}o)_2$. If the experimental conditions were made quite drastic, e. g., by using a large excess of RSH compound and a high temperature, all three R groups were cleaved. The anomalous behavior of tri-α-naphthylbismuth towards thiophenol has been noted before (page 297).

Kharasch (69a) has reported a method for the preparation of water-soluble organobismuth compounds of the general formula $RBi(SeC_6H_4COOH\text{-}o)_2$ by cleavage of R_3Bi compounds by selenosalicylic acid. This again demonstrates the unusual tendency to cleave two R groups.

In the course of a related study, triphenylbismuth was cleaved with several aliphatic and aromatic carboxylic acids (55). With benzoic and cinnamic acids the products were $C_6H_5Bi(OOCC_6H_5)_2$ and $C_6H_5Bi(OOCCH{=}CHC_6H_5)_2$, respectively, indicating again preferential cleavage of two R groups. With acetic, trichloroacetic, propionic, butyric, thioglycolic, lactic, salicylic, and thiosalicylic acids the cleavage products were mixtures which could not be resolved because of the insolubility of these salts in water and organic solvents. Koton (71) has described the complex products obtained by the cleavage of triphenylbismuth by formic and acetic acids when a large excess of these acids was employed.

It is of interest to point out that every compound possessing a carbon–bismuth-sulfur linkage which has been prepared is deep yellow in color. The products obtained with the carboxylic acids were always white powders.

(b) Pentavalent compounds

Pentavalent bismuth salts of the general formula $R_3Bi(OOCR)_2$ have been prepared by various methods (15, 19, 27, 111, 121).

TABLE 4

Organobismuth salts of organic acids

COMPOUND	MELTING POINT	REFERENCES
	°C.	
Trivalent compounds:		
$C_6H_5Bi(SC_6H_5)_2$	170 d.*	(55)
p-$ClC_6H_4Bi(SC_6H_5)_2$	170 d.	(55)
p-$CH_3C_6H_4Bi(SC_6H_5)_2$	155 d.	(55)
$C_6H_5Bi(SC_6H_4COOCH_3$-$o)_2$	102	(55)
Pentavalent compounds:		
Triphenylbismuth diacetate	162	(15, 19, 111, 121)
Triphenylbismuth diacetate·$0.5CH_3COOH$	137	(19)
Tri-*m*-tolylbismuth diacetate	149	(111)
Tri-*p*-tolylbismuth diacetate	162	(111)
Triphenylbismuth dibenzoate	173	(27)
Tri-*p*-tolylbismuth dibenzoate	169 d.	(55)
Tri-*o*-tolylbismuth disalicylate·C_6H_6	165 d.	(55)
Diphenylbiphenylbismuth dibenzoate	147	(52)
Diphenyl-β-naphthylbismuth dibenzoate	140	(52)
Triphenylbismuth dicinnamate	178	(55)
Triphenylbismuth disalicylate	185 d.	(55)
Triphenylbismuth di-*o*-carboxybenzoate	169 d.	(55)
Triphenylbismuth di-*p*-hydroxybenzoate	Above 250	(55)
Triphenylbismuth di-*p*-aminobenzoate	148 d.	(55)
Triphenylbismuth di-*p*-aminobenzoate·$2CH_3COCH_3$	148 d.	(55)
Triphenylbismuth dianthranilate·C_6H_6	102 d.	(55)
Triphenylbismuth dichloroacetate	156	(55)
Tri-*p*-chlorophenylbismuth disalicylate	187 d.	(55)
Triphenylbismuth dilactate		(19)
Triphenylbismuth dicamphorsulfonate		(19)
Triphenylbismuth ditartrate		(15)
Triphenylbismuth dipicrate		(15)

* d. = decomposes.

$$(1)\quad R_3Bi(OH)_2 + 2RCOOH \rightarrow R_3Bi(OOCR)_2 + 2H_2O$$

$$(2)\quad R_3Bi(OH)Cl + 2RCOOH \rightarrow R_3Bi(OOCR)_2 + H_2O + HCl$$

$$(3)\quad R_3BiCO_3 + 2RCOOH \rightarrow R_3Bi(OOCR)_2 + H_2O + CO_2$$

$$(4)\quad R_3BiX_2 + 2RCOOM \rightarrow R_3Bi(OOCR)_2 + 2MX$$

$$(5)\quad R_3Bi + (C_6H_5CO)_2O_2 \rightarrow R_3Bi(OOCC_6H_5)_2 + O_2$$

Reaction 3 has been employed to prepare a large number of pentavalent derivatives in which the benzoyl radical possessed potential water-solubilizing groups.

In an attempt to prepare amine hydrochlorides of triphenylbismuth dianthranilate or triphenylbismuth di-*p*-aminobenzoate the only products obtained were triphenylbismuth dichloride and the hydrochloride of the corresponding acid. With the disalicylate, di-*p*-hydroxybenzoate, and di-*o*-carboxybenzoate there occurred complete dissociation into triphenylbismuth dihydroxide and the potassium salt of the acid when the compounds were treated with dilute potassium hydroxide solution.

Proof of the structure of these pentavalent compounds was achieved by cleavage with hydrochloric acid and the identification of the R_3BiCl_2 and organic acid formed.

A rather interesting observation made during the course of this work was that when these derivatives were crystallized from acetone or benzene there was a general tendency to add molecules of solvent of crystallization. In several instances, as with tri-*o*-tolylbismuth disalicylate and triphenylbismuth dianthranilate, any attempts to remove the solvent resulted in the decomposition of the compound.

TABLE 5

Inorganic halide double salts of organobismuth compounds

COMPOUND	MELTING POINT	REFERENCES
	°C.	
(p-$CH_3C_6H_4)_3Bi\cdot HgCl_2$	234	(108)
(o-$CH_3C_6H_4)_3Bi\cdot HgCl_2$	148	(108)
(m-$(CH_3)_2C_6H_3)_3Bi\cdot HgCl_2$	160	(108)
(α-$C_{10}H_7)_3Bi\cdot HgCl_2$	193	(108)
$(CH_3)_2BiBr\cdot ZnBr_2$		(80)

Table 4 lists all the known organobismuth salts of organic acids, both tertiary and pentavalent types. Table 5 lists the comparatively few inorganic halide double salts of organobismuth compounds.

D. ANALYTICAL PROCEDURES

1. *Qualitative*

A very convenient test for bismuth in an organobismuth compound is to burn a small portion of the compound on a clean spatula. The yellow-brown residue of bismuth oxide is easily recognized. Another method involves cleaving 0.05 g. of the compound with 5 ml. of concentrated nitric acid and heating the solution to dryness. After allowing the residue to cool, it is taken up in 5 ml. of concentrated hydrochloric acid, concentrated to 1 ml., and then treated with a freshly prepared solution of sodium stannite. A heavy black precipitate of metallic bismuth forms, if bismuth is present in the compound.

The color test described on page 306 also possesses possible utilization as a qualitative test for the presence of some organobismuth compounds.

2. *Quantitative*

Michaelis and Polis (84) have described the determination of bismuth in organobismuth compounds as bismuth trioxide and Challenger and Goddard (19) as bismuth trisulfide. The analysis as the phosphate appears to be superior to both these methods (53).

III. THE PHYSICAL PROPERTIES OF ORGANOBISMUTH COMPOUNDS

A. MOLECULAR STRUCTURE

Bergmann and Schütz (4) have found the dipole moment of triphenylbismuth to be zero. From this they have concluded that its structure is that of a plane triangle with the bismuth atom situated at the center of gravity. The triphenylphosphines, -amines, -arsines, and -stibines appear to be triangular pyramids. Pai (91) and Rosenbaum, Rubin, and Sandberg (99a), from studies of the Raman spectra of trimethylbismuth, have concluded that this compound possesses a pyramidal structure, the three methyl groups forming the triangular base and the bismuth atom the apex.

B. CRYSTAL STRUCTURE

The crystal structures of triphenylbismuth (2), triphenylbismuth difluoride (26), and diphenylbismuth thiocyanate (26) have been studied. Michaelis and Marquardt (83) have reported two crystal forms of triphenylbismuth, one melting at 75°C. and the other at 78°C. This has not been substantiated by other workers in this field. Drew and Landquist (33) and Pascal (95) have studied the triphenyl compounds of the elements of Group V for possible isomorphism. They have observed compatibility only with triphenylphosphorus and triphenylarsenic. No close relationship such as can be found in the organometallic compounds of the elements of Group IV exists among the organometallic compounds containing Group V elements.

Greenwood (60, 61) and Bryant (9) have studied the rotatory polarization of light by crystals of triphenylbismuth dichloride.

From x-ray studies, Greenwood (61) has data which would indicate that triphenylbismuth dichloride has eight molecules in a unit cell.

C. PHENOMENA WITH LIGHT

Schaefer and Hein (101) have studied the absorption of light by trimethylbismuth, triphenylbismuth, and triphenylbismuth dichloride, and have observed that the first two compounds show normal curves and obey Beer's law. The dichloride absorbs light much more strongly than the tertiary compounds, owing to the two chlorine atoms and the higher valence of bismuth.

IV. THE BIOLOGICAL APPLICATIONS OF ORGANOBISMUTH COMPOUNDS

A. ORGANOBISMUTH THERAPY

The therapeutic properties of bismuth were recognized as early as 1660. By 1750 it had been used in the treatment of gonorrhea and by 1779 in syphilis therapy. It was not until 1921, however, that research in the field of bismuth therapy became intense. With the pioneer work of Levaditi and his coworkers there began what may be called "The French School of Bismuth Therapy." In the six years immediately following Levaditi's first publication, nineteen hundred and sixteen articles on bismuth therapy were published, and by 1935, a conservative estimate would have placed this number close to five thousand. By 1932 about two hundred and fifty organic and inorganic bismuth compounds had been prepared and investigated for possible therapeutic utilization.

One result of this intensive research has been the large number of contradictory reports to be found with regards to the effectiveness of bismuth therapy. The actual value of organic bismuth compounds in spirochetosis has long been the subject of debate, although their value in the intermittent treatment of syphilis by arsenicals is acknowledged. Bismuth is much more toxic than arsenic and acts more slowly. Its compounds can be injected intramuscularly only, since intravenously their therapeutic index is about 1:3. This is true even for those compounds accepted as new and non-official remedies by the Council on Pharmacy and Chemistry of the American Medical Association. According to Dr. R. A. Vonderlehr, Assistant Surgeon General, Division of Venereal Diseases of the United States Public Health Service (116), " . . . It is the general consensus of opinion of experts in this country and particularly of the Coöperative Clinical Group that when all factors are taken into consideration, bismuth subsalicylate suspended in oil is probably the most practical bismuth preparation (for the treatment of syphilis)."

When injected intramuscularly, bismuth compounds form "depots" which slowly release bismuth into the blood. In some instances absorption is so slow that there is still evidence of bismuth at the point of injection after 400 days.

Kolle (70) has found that such a depot in the ear of a rabbit will prevent the appearance of scrotal lesions following intratesticular injections of the syphilis virus. When the ear was amputated after 3 months, however, scrotal lesions developed after an interval of several weeks. This can mean perhaps that the virulent organisms were not destroyed by the bismuth but that their multiplication was inhibited. It is to be noted, however, that *complete* cures of syphilis by the exclusive use of organic bismuth compounds have been reported also.

Of the more than five thousand papers published in the field of bismuth therapy between 1921 and 1935, only a very few have been concerned with the use of organobismuth compounds. Giemsa (39, 40, 41, 42) reported the remarkable healing properties of triphenylbismuth in experimental canine syphilis, relapsing fever, and nagana. He proposed its use in the later stages of syphilis because of its lipoid solubility, which enables it to penetrate undecomposed into the central nervous system. Albrecht and Evers (1) and Schlossberger (102) were unable to confirm these favorable reports. Rothermundt and Wichmann (99) have reported that triphenylbismuth had no therapeutic activity in the treatment of mice infected with *S. crocidurae* or *S. hispanica*. Sollman and Seifter (107) have ingeniously tested some alkylbismuth compounds for their antisyphilitic activity. They found that trimethylbismuth could be handled safely and was sufficiently soluble in water to produce marked effects in experimental animals when treatment was by vein, inhalation, or skin. It had a definite healing effect in rabbit syphilis, but it demonstrated no extraordinary therapeutic activity. The higher alkyls, trilaurylbismuth, tricetylbismuth. and cetylbismuth dibromide, were poorly absorbed and inactive.

Walker (117) has determined the least concentration of four organobismuth compounds which would destroy *Colpidum colpoda* in 3 min. His results are as follows:

Triphenylbismuth	mole/11,000
Diphenylbismuth bromide	mole/270,000
Tri-*p*-tolylbismuth	mole/2000
Triphenylbismuth dibromide	mole/7100

From this, one may observe that the secondary derivative, diphenylbismuth bromide, possesses the highest effectiveness. This holds true generally, inasmuch as $(C_6H_5)_2SbX$ and $(C_6H_5)_2AsX$ (where X is any halogen) are more effective than the primary or tertiary derivatives of the same elements. Lecoq (74) has found $C_6H_5AsCl_2$ to be more toxic than either $C_6H_5SbCl_2$ or $C_6H_5BiCl_2$. Pentavalent derivatives in this family are weakly toxic, and it has been suggested that whatever toxic effects they possess is due to their first being reduced to the trivalent form.

It is apparent that little work has been done in bismuth therapy with organobismuth compounds. The principal objection seems to be their high toxicity. If organobismuth compounds of low toxicity could be synthesized, a more accurate estimation of their effectiveness would be possible.

B. ABSORPTION OF BISMUTH BY THE ORGANISM

If massage of the muscle follows an intramuscular injection of a bismuth compound, bismuth appears in the blood 2 hr. after the injection. Without massage, the appearance is delayed for 24 hr. The concentration of bismuth appears to be highest in the kidneys, and less in the liver, spleen, and lungs. The blood and bile show varying content. Some of the bismuth may be stored in the liver and other organs, but the greater part is eliminated by the kidneys, liver, and intestinal mucosa. It appears in the urine from 18 to 24 hr. after the injection.

C. PHYSIOLOGICAL ACTION OF BISMUTH

Following an intramuscular injection of a bismuth compound there occurs a local necrosis of the muscle fibre which is slowly absorbed and replaced by scar tissue. Sometimes sterile abscesses are formed. The gluteal muscle mass is large enough to tolerate from fifty to a hundred or more injections without serious difficulty.

In the bismuth poisoning of animals by a single large dose, the chief pathological changes occur in the kidney and liver, other organs being affected little or not at all. In the kidneys there are marked tubular nephritis and degenerative changes similar to those produced by mercury poisoning. Intravenous injections produce these alterations more frequently than do intramuscular administrations. Of the local reactions, pain is the most frequent and this is due, in part, to the vehicle. A compound in olive oil may be extremely painful; in peanut oil it is bland and non-irritating.

The administration of bismuth compounds in therapeutic doses at weekly intervals is, however, remarkably free from toxic effects. The one exception appears in the bluish-gray pigment line which forms on the gum margins of the mouth after a half-dozen injections. In rare instances (thirty-five in the period from 1922 to 1935) death may result from bismuth therapy, but these cases have been reported as due to accidental intravenous injection.

V. Summary

An attempt has been made in the preceding review to survey the literature on organobismuth compounds up to April, 1942. Those properties which are characteristic of organobismuth compounds have been stressed, and some attention has been directed towards correlating the organometallic compounds of arsenic, antimony, and bismuth, in order to show gradations of stability, reactivity, and compound formation.

REFERENCES

Organobismuth compounds

(1) Albrecht, B., and Evers, E.: Med. Klin. **21**, 1807 (1925).
(2) Arzruni: Ann. **251**, 326 (1889).
(3) Bart, H.: Ann. **429**, 55 (1922); German patent 250,264 (August 1, 1910); Chem. Zentr. **83**, II, 882 (1912).
(4) Bergmann, E., and Schütz, W.: Z. physik. Chem. **B19**, 401 (1932).
(5) Birckenbach, L., and Kellermann, K.: Ber. **58**, 786 (1925).
(6) Birckenbach, L., and Kellermann, K.: Ber. **58**, 2377 (1925).
(7) Blicke, F. F., Oakdale, U. O., and Smith, F. D.: J. Am. Chem. Soc. **53**, 1025 (1931).
(8) Breed: Ann. **82**, 106 (1852); J. prakt. Chem. **56**, 341 (1852).
(9) Bryant, W. M. D.: Am. Mineral. **20**, 281 (1935).
(10) Cambi, L.: Atti accad. Lincei **21**, I, 773 (1912).
(11a) Calingaert, G., Soroos, H., and Hnizda, V.: J. Am. Chem. Soc. **62**, 1107 (1940).
(11b) Calingaert, G., and Beatty, H. A.: J. Am. Chem. Soc. **61**, 2748 (1939).
(11c) Calingaert, G., Soroos, H., and Thomson, G. W.: J. Am. Chem. Soc. **62**, 1542 (1940).
(12) Caplin, W.: Thesis, Iowa State College, 1934.
(13) Challenger, F.: Proc. Chem. Soc. **29**, 76 (1913).
(14) Challenger, F.: J. Chem. Soc. **105**, 2210 (1914).
(15) Challenger, F. J. Chem. Soc. **109**, 250 (1916).
(16) Challenger, F. and Allpress, C. F.: Proc. Chem. Soc. **30**, 293 (1914).
(17) Challenger, F. and Allpress, C. F.: J. Chem. Soc. **107**, 16 (1915).
(18) Challenger, F. and Allpress, C. F.: J. Chem. Soc. **119**, 913 (1921).
(19) Challenger, F. and Goddard, A. E.: J. Chem. Soc. **117**, 762 (1920).
(20) Challenger, F. Peters, A. T., and Halévy, J.: J. Chem. Soc. **1926**, 1648.
(21) Challenger, F. and Pritchard, F.: J. Chem. Soc. **125**, 864 (1924).
(22) Challenger, F. and Richards, O. V.: J. Chem. Soc. **1934**, 405.
(23) Challenger, F. and Ridgway, L. R.: J. Chem. Soc. **121**, 104 (1922).
(24) Challenger, F. and Rothstein, E.: J. Chem. Soc. **1934**, 1258.
(25) Challenger, F., Smith, A. L., and Paton, F. J.: J. Chem. Soc. **123**, 1046 (1923).

(26) Challenger, F., and Wilkinson, J. F.: J. Chem. Soc. **121**, 91 (1922).

(27) Challenger, F., and Wilson, V. K.: J. Chem. Soc. **1927**, 209.

(28) Classen, A., and Ney, O.: Ber. **53**, 2267 (1920).

(29) Classen, A., and Ney, O.: Z. anorg. allgem. Chem. **115**, 253 (1921).

(30) Classen, A., and Strauch, G.: Z. anorg. allgem. Chem. **141**, 82 (1924).

(31) Davies, W. C., Norvick, I., and Jones, W. J.: Bull. soc. chim. [4] **49**, 187 (1931).

(32) Denham, H. G.: J. Am. Chem. Soc. **43**, 2367 (1921).

(33) Drew, H. D. K., and Landquist, J. K.: J. Chem. Soc. **1935**, 1480.

(34) Dünhaupt, F.: J. prakt. Chem. **61**, 399 (1854).

(35) Ehrlich, P., and Karrer, P.: Ber. **46**, 3564 (1913).

(36) Frankland, E., and Duppa, B. F.: J. Chem. Soc. **17**, 29 (1864).

(37) Fr. Fabrykant: Bull. pharm. Inst. Poland, No. **2**, I (1927); Chem. Abstracts **23**, 118 (1929).

(38) Ganassini, D., and Santi, U.: Boll. chim. farm. **64**, 289 (1925); Chem. Abstracts **20**, 899 (1926).

(39) Giemsa, G.: Dermatol. Wochschr. **76**, 523 (1923).

(40) Giemsa, G.: Dermatol. Wochschr. **79**, 1567 (1924).

(41) Giemsa, G.: Z. angew. Chem. **37**, 765 (1924).

(42) Giemsa, G.: Med. Klin. **22**, 60 (1926).

(43) Gillmeister, A.: Ber. **30**, 2843 (1897).

(44) Gilman, H.: *Organic Chemistry*, Vol. I, p. 479. John Wiley and Sons, Inc., New York (1938).

(45a) Gilman, H., and Jones, R. G.: J. Am. Chem. Soc. **62**, 2353 (1940).

(45b) Gilman, H., and Jones, R. G.: J. Am. Chem. Soc. **63**, 1162 (1941).

(45c) Gilman, H., and Jones, R. G.: J. Am. Chem. Soc. **62**, 1243 (1940).

(46) Gilman, H., and Moore, F. W.: J. Am. Chem. Soc. **62**, 1843 (1940).

(47) Gilman, H., and Nelson, J. F.: J. Am. Chem. Soc. **59**, 935 (1937).

(48) Gilman, H., and Svigoon, A. C.: J. Am. Chem. Soc. **61**, 3586 (1939).

(49) Gilman, H., and Yablunky, H. L.: J. Am. Chem. Soc. **62**, 665 (1940).

(50) Gilman, H., and Apperson, L. D. (section by H. L. Yablunky): J. Org. Chem. **4**, 162 (1939).

(51) Gilman, H., and Yablunky, H. L.: J. Am. Chem. Soc. **63**, 207 (1941).

(52) Gilman, H., and Yablunky, H. L.: J. Am. Chem. Soc. **63**, 212 (1941).

(53) Gilman, H., and Yablunky, H. L.: J. Am. Chem. Soc. **63**, 949 (1941).

(54) Gilman, H., Yablunky, H. L., and Svigoon, A. C.: J. Am. Chem. Soc. **61**, 1170 (1939).

(55) Gilman, H., and Yablunky, H. L.: Unpublished data.

(56) Gilman, H., and Yablunky, H. L.: J. Am. Chem. Soc. **63**, 839 (1941).

(57) Goddard, A. E.: J. Chem. Soc. **121**, 36 (1922).

(58) Goddard, A. E., Ashley, J. N., and Evans, R. B.: J. Chem. Soc. **121**, 978 (1922).

(59) Goddard, D., and Goddard, A. E.: J. Chem. Soc. **121**, 256 (1922).

(60) Greenwood, G.: Mineralog. Mag. **20**, 123 (1923).

(61) Greenwood, G.: Am. Mineral. **16**, 473 (1931).

(62) Grüttner, G.: Ber. **47**, 3257 (1914).

(63) Grüttner, G., and Wiernik, M.: Ber. **48**, 1473 (1915).

(64) Hasenbaümer, J.: Ber. **31**, 2910 (1898).

(65a) Hein, F., Petzchner, E., Wagler, K., and Segitz, F. A.: Z. anorg. allgem. Chem. **141**, 161 (1924).

(65b) Hein, F., and Segitz, F. A.: Z. anorg. allgem. Chem. **158**, 153 (1926).

(66) Hilpert, S., and Ditmar, M.: Ber. **46**, 3738 (1913).

(67) Hilpert, S., and Grüttner, G.: Ber. **46**, 1675 (1913).

(68) Ipatieff, W. and Razuvaev, G.: Ber. **63**, 1110 (1930). See, also, Ipatieff, W.: *Catalytic Reactions at High Pressures and Temperatures*, pp. 358–63. The Macmillan Company, New York (1936).

(69a) KHARASCH, M. S.: U. S. patent 1,858,958 (Chem. Abstracts **26**, 3806 (1932)).

(69b) KHARASCH, M. S., LEGAULT, R. R., AND SPROWLS, W. R.: J. Org. Chem. **3**, 409 (1938), and earlier papers.

(69c) KHARASCH, M. S., AND SWARTZ, S.: J. Org. Chem. **3**, 405 (1938).

(70) KOLLE, W.: Med. Klin. **20**, 1097 (1924).

(71) KOTON, M. M.: J. Gen. Chem. (U.S.S.R.) **9**, 2283 (1939); Chem. Abstracts **34**, 5049 (1940). See, also, KOTON, M. M.: J. Gen. Chem. (U.S.S.R.) **11**, 379 (1941); Chem. Abstracts **35**, 5870 (1941).

(72) KRAUSE, E., AND RENWANZ, G.: Ber. **62**, 1710 (1929).

(73) KRAUSE, E., AND RENWANZ, G.: Ber. **65**, 777 (1932).

(74) LECOQ, H.: J. pharm. Belg. **19**, 133 (1937).

(75) LEIGH-SMITH, A., AND RICHARDSON, H. O. W.: Nature **135**, 828 (1935).

(76) LÖWIG, C., AND SCHWEIZER, E.: Ann. **75**, 355 (1850).

(77) MAKAROVA, L. G.: J. Gen. Chem. (U.S.S.R.) **7**, 143 (1937).

(78) MAKAROVA, L. G., AND NESMEYANOV, A. N.: J. Gen. Chem. (U.S.S.R.) **9**, 771 (1939); Chem. Abstracts **34**, 391 (1940).

(79) MARPLE, K.: Thesis, Iowa State College, 1935, p. 16.

(80) MARQUARDT, A.: Ber. **20**, 1516 (1887).

(81) MARQUARDT, A.: Ber. **21**, 2035 (1888).

(82) MICHAELIS, A.: Ber. **20**, 52 (1887).

(83) MICHAELIS, A., AND MARQUARDT, A.: Ann. **251**, 323 (1889).

(84) MICHAELIS, A., AND POLIS, A.: Ber. **20**, 54 (1887).

(85) MICHAELIS, A., AND REESE, A.: Ber. **15**, 2877 (1882).

(86) MICHAELIS, A., AND REESE, A.: Ann. **233**, 39 (1886).

(87) MORTENSEN, R. A., AND LEIGHTON, P. A.: J. Am. Chem. Soc. **56**, 2397 (1934).

(88) MOUREU, C., DUFRAISSE, C., AND BADOCHE, M.: Compt. rend. **187**, 1092 (1928).

(89) NELSON, J.: Thesis, Iowa State College, 1937, pp. 66–70.

(90) NORVICK, I.: Nature **135**, 1038 (1935).

(91) PAI, N. G.: Proc. Roy. Soc. (London) **A149**, 29 (1935).

(92) PANETH, F., AND HOFEDITZ, W.: Ber. **62**, 1335 (1929).

(93) PANETH, F. A., AND LOLEIT, H.: J. Chem. Soc. **1935**, 366.

(94) PANETH, F.: Trans. Faraday Soc. **30**, 179 (1934).

(95) PASCAL, P.: Bull. soc. chim. [4] **11**, 595 (1912).

(96) PASCAL, P.: Compt. rend. **156**, 1904 (1913).

(97) PFEIFFER, P., AND PIETSCH, H.: Ber. **37**, 4620 (1904).

(98) RICE, F. O., AND COWORKERS: See RICE, F. O., AND RICE, K. K.: The Aliphatic Free Radicals, p. 58. John Hopkins University Press, Baltimore (1935).

(99a) ROSENBAUM, E. J., RUBIN, D. J., AND SANDBERG, C. R.: J. Chem. Phys. **8**, 366 (1940).

(99b) ROTHERMUNDT, M., AND WICHMANN, F.: Z. Immunitäts. **77**, 1 (1932).

(100) ROZENBLUMÓWNA, ST., AND WEIL, ST.: Bull. trav. inst. pharm. etat (Warsaw) **1927**, No. 1, 3; Chem. Abstracts **21**, 1449 (1927).

(101) SCHAEFER, K., AND HEIN, F.: Z. anorg. allgem. Chem. **100**, 249 (1917).

(102) SCHLOSSBERGER, H.: Z. Hyg. Infektionskrankh. **108**, 627 (1928).

(103) SCHLENK, W., AND HOLTZ, J.: Ber. **49**, 603 (1916).

(104) SCHLENK, W., AND HOLTZ, J.: Ber. **50**, 274 (1917).

(105) SEIFTER, J.: Private communication.

(106) SMITH, R. H., AND ANDREWS, D. H.: J. Am. Chem. Soc. **53**, 3661 (1931).

(107) SOLLMAN, T., AND SEIFTER, J.: J. Pharmacol. **67**, 17 (1939).

(108) STILP, K.: Inaugural Dissertation, University of Rostock, 1910.

(109) SUPNIEWSKI, J. V.: Roczniki Chem. **5**, 298 (1925).

(110) SUPNIEWSKI, J. V.: Roczniki Chem. **6**, 97 (1926).

(111) SUPNIEWSKI, J. V., AND ADAMS, R.: J. Am. Chem. Soc. **48**, 507 (1926).

(112) SVIGOON, A. C.: Thesis, Iowa State College, 1939.

(113) TALALAEVA, T. V., AND KOCHESHKOV, K. A.: J. Gen. Chem. (U.S.S.R.) **8**, 1831 (1938); Chem. Abstracts **33**, 5819 (1939).

(114) VOLMAR AND CHARDEYRON: Bull. soc. chim. [4] **31**, 545 (1922).

(115) VORLÄNDER, D.: Ber. **58**, 1893 (1925).

(116) VONDERLEHR, R.: Private communication.

(117) WALKER, E.: Biochem. J. **22**, 292 (1928).

(118) WILKINSON, J. F., AND CHALLENGER, F.: J. Chem. Soc. **125**, 854 (1924).

(119) WINMILL, T. F.: J. Chem. Soc. **101**, 718 (1912).

(120) WORRALL, D. E.: J. Am. Chem. Soc. **58**, 1820 (1936).

(121) ZHITKOVA, L. A., SHEVERDINA, N. I., AND KOCHESHKOV, K. A.: J. Gen. Chem. (U.S.S.R.) **8**, 1839 (1938); Chem. Abstracts **33**, 5819 (1939).

General references

CHALLENGER, F.: "Organic Compounds of Bismuth", in Thorpe and Whiteley's *Dictionary of Applied Chemistry*, 4th edition, pp. 701-2. Longmans, Green and Company, London (1937). See, also, CHALLENGER, F.: *The Industrial Chemist* **3**, 125 (1927).

GARZULY, R.: "Organometalle," in Vol. XIX of Herz' *Sammlung chemisch und chemisch-technischer Vorträge*, pp. 414-22. Enke, Stuttgart (1927).

GILMAN, H.: *Organic Chemistry*, pp. 474-5. John Wiley and Sons, Inc., New York (1938).

GODDARD, D.: "Organometallic Compounds," in Vol. XI, Part 3, of Friend's *A Text-Book of Inorganic Chemistry*, pp. 265-86. Charles Griffin Company, London (1936).

KLARMANN, E.: "Darstellung metallorganischer Verbindungen," in Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Abt. 1, Chemische Methoden, Teil 2, 2 Hälfte, Heft 4, pp. 2288-94. Urban und Schwarzenberg, Berlin (1933).

KRAUSE, E., AND VON GROSSE, A.: *Die Chemie der metall-organischen Verbindungen*, pp. 641-53. Gebrüder Borntraeger, Berlin (1937).

SCHMIDT, J.: *Organometallverbindungen*, Part 2, pp. 328-31, 283. Wissenschaftliche Verlagsgesellschaft, m.b.H. (1934).

Bismuth therapy

COLE: "The Use of Bismuth in the Treatment of Syphilis," *Venereal Disease Information* **12**, No. 4, 1 (1931).

DALE, H.: Physiol. Rev. **3**, 359 (1923).

FINDLAY, G. M.: *Recent Advances in Chemotherapy*, pp. 373-93, 423. P. Blakiston's Son and Company, Philadelphia (1930).

FISCHL, V., AND SCHLOSSBERGER, H.: *Handbuch der Chemotherapie*, pp. 612-27. Fischer's Medizinische Buchhandlung, Leipzig (1934).

FORST, A.: "Wismut," in Vol. III, Part 4, of Heffter's *Handbuch der experimentellen Pharmakologie*, pp. 2249-730. Julius Springer, Berlin (1935). This is the most comprehensive treatise on this subject. The literature on bismuth therapy is covered up to 1935.

Merck's Index, 5th edition, pp. 82-8. Merck & Co., Inc., Rahway, New Jersey (1940).

MOORE, J.: *The Modern Treatment of Syphilis*, pp. 110-28. Charles C. Thomas, Springfield, Illinois, and Baltimore (1935).

ORGANOARSENIC AND ORGANOANTIMONY COMPOUNDS

General references

CHRISTIANSEN, W. G.: *Organic Derivatives of Antimony.* The Chemical Catalog Company, Inc., New York (1925).

GODDARD, D.: "Organometallic Compounds," in Vol. XI, Part 3, of Friend's *A Text-Book of Inorganic Chemistry*, pp. 166-262. Charles Griffin Company, London (1936).

MORGAN, G. T.: *Organic Compounds of Arsenic and Antimony.* Longmans, Green and Company, London (1918).

RAIZISS, G. W., AND GAVRON, J. L.: *Organic Arsenical Compounds.* The Chemical Catalog Company, Inc., New York (1923).

SYMPOSIUM ON PHYSICOCHEMICAL METHODS IN PROTEIN CHEMISTRY[1]

INTRODUCTION TO THE SYMPOSIUM

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Received March 26, 1942

If at any time the progress in the direction of the understanding of the chemistry and physics of proteins appears to be slow it may be well to recall that, as late as twenty years ago, there was a school of physical chemists which held that proteins could be regarded as structureless continua the properties of which were considered to be determined by the state of dispersion and by materials adsorbed on the surfaces produced by such dispersion. The impossibility of holding such an opinion at the present time is a rough measure of the advance in this field. While it is true that no single fundamental problem concerned with proteins has been finally solved, the tools with which such solutions will finally be attained may very well be in hand. The following papers, which are the result of a symposium held at a meeting of the American Chemical Society at Atlantic City on September 9, 1941, illustrate the diversity and the power of the physicochemical methods now available. One of the first breaks with the older "colloidal" conceptions was that produced by Sørensen and by Loeb when they showed that proteins can combine stoichiometrically with acids and bases. Svedberg and his school have shown that proteins have definite molecular weights. Tiselius has demonstrated that proteins have reproducible electrophoretic mobilities at given pH values and ionic strengths. It has been shown by Northrop and his associates that these substances have definite solubilities and are thus components in the sense of the phase rule. The diffusion measurements of Lamm are also consistent with the assumption that proteins are large molecules of definite molecular weight. To interpret the results of dielectric-constant measurements, and with the ultracentrifuge, the shapes of the protein molecules must be considered. Improved analytical methods, due largely to Bergmann and to Van Slyke and their associates, have demonstrated that proteins have definite chemical compositions.

One result of the use of these physicochemical methods is the ever-increasing number of proteins that can be proved by all the tests available to be definite chemical substances. The importance of these pure proteins to chemistry and physiology can hardly be overestimated.

In addition to the papers published in this number of *Chemical Reviews*, papers were also presented at the symposium by Dr. David Rittenberg on the investigation of the composition of proteins by means of isotopic tracers, and by Prof. W. D. Harkins and Dr. G. E. Boyd on the study of proteins with surface films. It is to be regretted that the defense researches of these authors have made it impossible to prepare these papers for publication.

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[1] This Symposium was held under the joint auspices of the Division of Physical and Inorganic Chemistry and the Division of Biological Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September 8–12, 1941.

RECENT ADVANCES IN THE STUDY OF PROTEINS BY ELECTROPHORESIS[1]

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Recent improvements in the experimental procedure for electrophoretic analysis by the moving-boundary method are described. These include the new Tiselius cell and the schlieren methods now available for recording the complete electrophoretic pattern. With the aid of the new procedures a study has been made of the conditions under which satisfactory patterns of the proteins of human plasma may be obtained. Of the many buffer solutions tested as solvents for the plasma proteins, a 0.1 N sodium diethylbarbiturate solution having a pH of 8.6 at 25°C. gave the best patterns for electrophoretic analysis. Using a typical pattern obtained in this solvent as an example, the current methods of computing protein concentrations from the patterns have been critically examined and the assumptions that are necessary in making these computations have been stated. Moreover, from a consideration of the necessity of conserving matter in the transport of materials by electrophoresis it has been possible to derive relations that are of value in checking the consistency of the analyses and in testing the validity of one of the assumptions.

I. INTRODUCTION

Four years have elapsed since Tiselius (21) described his modification of the moving-boundary method for the electrophoresis of proteins and related materials. It is one of the purposes of this report to describe the method in its present form, including recent improvements in the experimental procedure. Moreover, the author will take this opportunity to include details of manipulation and procedure which have hitherto escaped publication.

Since its introduction in 1937 the method has been used for the study of a variety of problems, particularly for the electrophoretic analysis of naturally occurring protein mixtures. Human plasma is an important example of such a mixture, and the analysis of this material has recently been improved by a study, the results of which are also reported in this paper, of the conditions under which satisfactory patterns may be obtained. Moreover, an interpretation of the patterns of protein mixtures is suggested, and the current methods of computing concentrations from the patterns are critically examined.

II. THE NEW TISELIUS ELECTROPHORESIS CELL

In the moving-boundary method for the electrophoresis of proteins a sharp boundary between the protein and buffer solutions is formed initially in each of the two sides of a U-shaped channel, with the denser protein solution underneath.

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[1] Presented at the Symposium on Physicochemical Methods in Protein Chemistry, which was held under the joint auspices of the Division of Physical and Inorganic Chemistry and the Division of Biological Chemistry at the 102nd meeting of the American Chemical Society, Atlantic City, New Jersey, September 8-12, 1941.

The latest cell devised by Tiselius (5, 20) for this purpose is shown in cross section in figure 1 and consists of the three sections I to III. These may be slid over one another along the planes *a-a′* and *b-b′*. Through the cell runs a U-shaped channel having a rectangular cross section of 3 x 25 mm. With the cell in place in its support, which will be shown below, the boundaries are formed as follows: The bottom section III is filled with the protein solution, the filling extending above *b-b′*, and the contents of this section are then isolated by displacement of this section to the left, as shown at A in figure 1. One side of section II is filled with protein solution and the other side rinsed (with the aid of a long stainless-steel needle attached to a syringe), and filled with buffer solution, both sides being filled to a level above *a-a′*. After connecting to the electrode vessels and partially filling the latter with buffer solution, the assembly is placed in a thermostat regulating at 0.5°C. When thermal equilibrium has been established in the channel, section II is displaced to the right, after which the bottom section is returned to the center, as shown in B of figure 1. The excess protein solution is then rinsed out of the top section and both sides of this section, together with the attached electrode vessels, are filled with buffer solution, as will be described below. Now by returning section II to the center position, figure 1 C, boundaries between the protein and buffer solutions are formed at the junction of sections I and II in one side of the channel and between sections II and III in the other side.

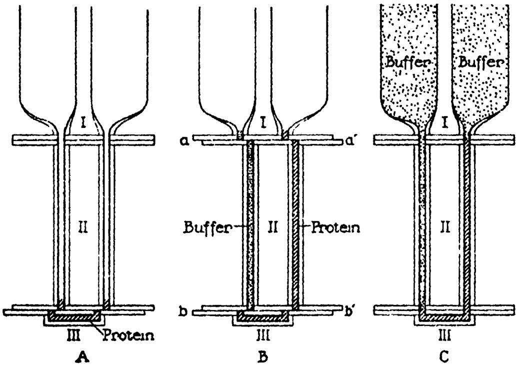

FIG. 1. Diagrams illustrating the initial formation of the boundaries in the Tiselius electrophoresis cell with the tall center section.

The support for the cell and the electrode vessels, together with the mechanism (5) for moving the sections of the cell in relation to each other, are shown in figure 2. With the electrophoresis cell filled as described, but before the boundaries have been formed, buffer solution is introduced into the electrode vessels to the levels *l-l′*. The silver–silver chloride electrodes E and E′ (6) are next inserted, care being taken not to trap air bubbles as the ground glass stopper f is seated. Concentrated potassium chloride is then introduced around each electrode by allowing 25 ml. of 1 *N* potassium chloride to flow slowly down each of the silver tubes t and t′. As the buffer solution around the electrode E, for example, is displaced, the liquid level rises in the hollow stopper p and the excess buffer solution overflows through c. The tube t is closed while still filled with solution and the capillary rubber tube c, also filled with solution, is then connected, as shown in figure 2, to the syringe d, the latter having been previously filled with buffer solution and clamped in position in the "compensator." The latter device, not shown in the figure, imparts, with the aid of a small synchronous motor, a uniform movement, at the desired rate, to the piston of the syringe. By turning the stopcock m clockwise through 90° from the position shown in figure 2, the right-hand side of the apparatus is closed, connection with the compensator being retained. It is essential that air bubbles be excluded from the closed side, and the manipulation outlined here achieves that purpose. The boundaries are then formed as has been described and are brought out from behind the opaque horizontal plates of the cell by slow injection of buffer from the syringe, after which the latter is isolated by turning the stopcock m through 45°. Application of a potential in the appropriate direction then causes, in general, the boundary in one side of the center section to rise and that in the other side to descend. If the protein solution contains several

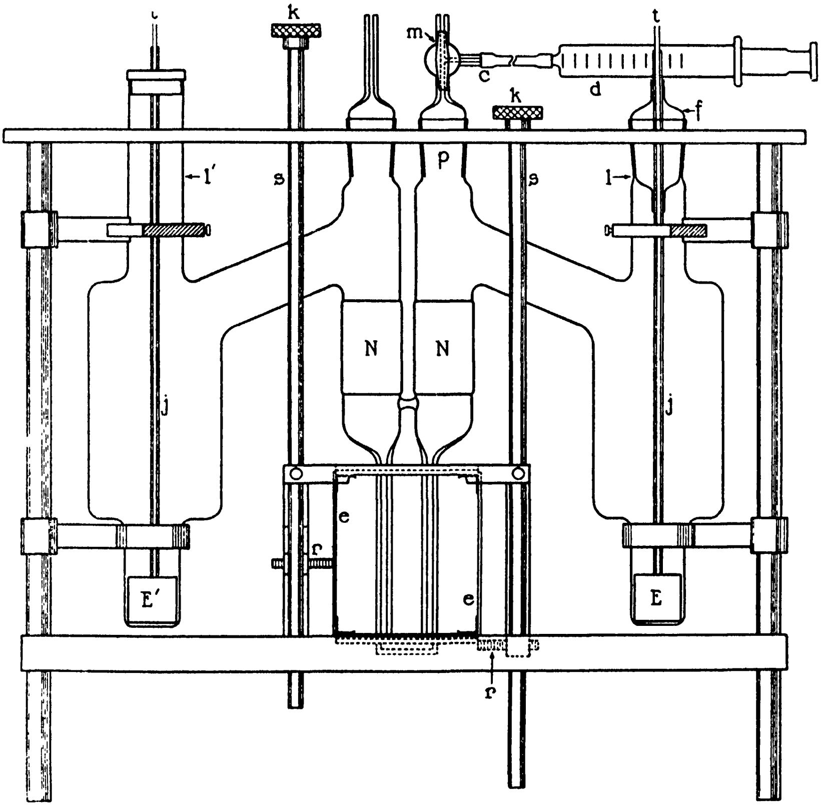

FIG. 2. Electrophoresis cell, electrode vessels, and support

components the mobilities of which differ sufficiently, the initially single boundary in each side of the channel will separate, on electrophoresis, into a number of separate boundaries exclusive of the δ and ϵ (7) effects, equal to the number of components in the mixture. In the electrophoresis of plasma at pH values above 7, all of the components are negatively charged. In this case the pattern of the rising boundaries may also be termed the anode or positive pattern, while that of the descending boundaries is the cathode or negative pattern.

The tall center section II of figure 1 is interchangeable with, and replaces, the two identical short center sections of the original Tiselius cell, thereby eliminating the horizontal glass plates obscuring the middle of the field. With the new section it is possible to spread the boundaries over more than twice the usual distance, with the possibility of disclosing more detail in the electrophoretic pattern. This is illustrated later in this paper, the patterns of a human plasma, as obtained in the short section, being shown in figure 5 and those of the same material in the new tall section in figure 6. Although some of the increased resolution apparent in figure 6 is due to the use of a different buffer solvent and a higher concentration of protein, the improvements of the patterns have, in part, been made possible by the use of the new type of center section. This new section is to be recommended for electrophoretic analyses and also for electrophoretic separations except in the case of a mixture of two components. In this latter case it is usually possible to fill a short compartment with a separated component, and isolation of this section then permits easy recovery of the material.

With mixtures of more than two components, however, it is usually not possible to fill a short compartment with a separated component before one of the boundaries has been drawn into the bottom section. Under these conditions the layer of solution containing the separated component may be recovered with the aid of the "convection-proof" pipet of Tiselius (22) or, as carried out in this laboratory, with a fine glass capillary attached to the compensator syringe. The tip of the capillary is lowered, with the aid of a rack and pinion, to the proper level in the channel and the desired solution withdrawn. The introduction of the capillary does not disturb the boundaries appreciably, and the entire procedure is subject to schlieren observation and control. The presence of the capillary in the channel does not interfere with the electrophoresis and it is thus possible to begin the withdrawal of material from between two boundaries soon after their separation has occurred. The two processes may then proceed simultaneously, thereby saving considerable time.

III. RECORDING THE COMPLETE ELECTROPHORETIC PATTERN

Probably the most important advance in electrophoresis procedure has been in the development of methods by means of which the complete electrophoretic patterns are obtained. The optical devices for this purpose are based upon an effect employed by Foucault and Toepler for testing lenses and called by the latter the "schlieren method," since it rendered small differences of refractive index visible as schlieren or shadows. As originally adapted by Tiselius, a boundary appeared in the focus of the schlieren camera as a dark band. This method, however, did not furnish information as to the variation of the refractive index in the boundary and was soon replaced by methods which yielded the complete electrophoretic pattern. The latter is a plot of the refractive-index gradient in a thin layer of solution in the channel as ordinate against the height of the layer as abscissa. One of the methods, the schlieren-scanning method, was developed by the author (2), while the other, the cylindrical-lens method, is due to Philpot (12) and has been modified by Svensson (18, 19). A pattern obtained with the aid of the latter method is shown in figure 3b, which also includes, for comparison, a pattern of the same material as given by the scanning procedure (figure 3a). Both the simple schlieren and the schlieren-scanning methods have been adequately described elsewhere (7) and will not be reviewed here.

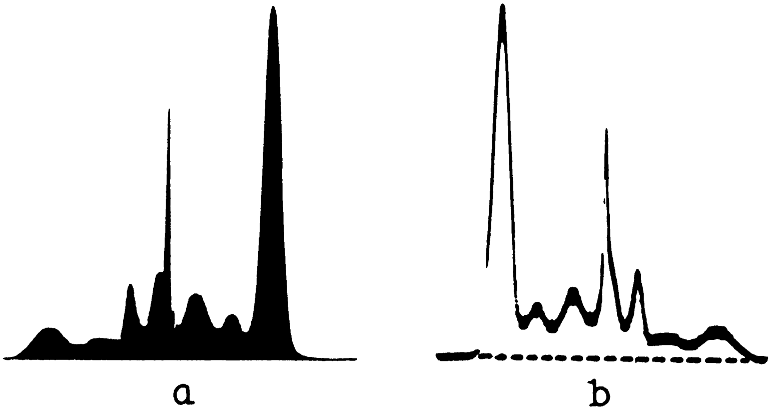

FIG. 3. Electrophoretic patterns of a human plasma obtained with the aid of (*a*) the schlieren-scanning method and (*b*) the cylindrical-lens method.

The cylindrical-lens method, however, affords an inexpensive and useful addition to the electrophoresis apparatus, and since the existing descriptions of its operation are not readily available to American chemists, a brief outline of this method will be presented here, with the aid of figure 4. An illuminated horizontal slit, present on the left of S but not shown in the figure, is focussed by means of the schlieren lens, S, in the plane of the schlieren diaphragm, D. The latter contains a diagonal slit, kk, as shown in the front view, D′. The camera objective, C, is focussed on the electrophoresis cell, E, and forms, in the absence of the lens H, a normal image of the cell on the ground-glass or photographic plate at G. The cylindrical lens, H,[2] with its axis vertical, is focussed on the schlieren diaphragm and also on the plate at G. Viewed from the side (figure 4a), the cylindrical lens has no effect on the pencils of light forming the cell image. Thus the vertical coördinate of each point in the image is conjugate to the corresponding level in the channel E and, owing to the focussing action of the camera lens, C, this also remains true for pencils that may be deflected by gradients in the channel. Viewed from above, however (figure 4b), the cylindrical lens, in conjunction with the diagonal slit, causes, as will be shown below, a lateral deviation of a pencil of light that is proportional to the vertical deflection the pencil has suffered in a boundary. The curve to the right of G in figure 4a represents the pattern of the boundary, B, as it would appear on the screen if the latter were hinged at the side and turned toward the reader, whereas if it were hinged at the top and turned, the pattern would appear as in figure 4b.

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[2] The objective C is a carefully corrected achromat of 2″ diameter and 36″ focal length. It is thus used at an aperture of *F*/18. The cylindrical lens H, on the other hand, is a single element lens of the same diameter but has a focal length of 16″ and hence works at the much higher aperture of *F*/8. Since this exceeds the maximum aperture of about *F*/16 usually considered safe (11) for photography with a simple lens, the use of the uncorrected cylindrical lens at an aperture of *F*/8 doubtless introduces aberrations in the patterns.

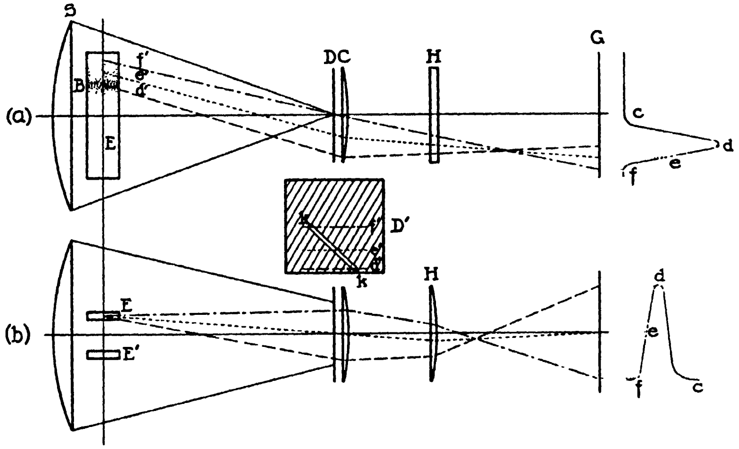

FIG. 4. Diagram of the cylindrical-lens method for the observation of the electrophoretic patterns.

If the fluid in the electrophoresis cell is homogeneous, all of the light through the channel is concentrated in an image of the illuminated slit at the upper or normal level on the diaphragm, i.e., f' of D', or D. As can be seen from the figure, only the extreme left-hand portion of the light in this image passes through the diaphragm to form a straight vertical line, i.e., the base line, on the screen at the position *c-f*. The width of this line varies with the width of the diagonal slit and, since a wide line is undesirable, Svensson has made the practical suggestion of tapering the end of the slit kk to a point.

If, on the other hand, a boundary B is present in the cell, a pencil through the layer of solution in the boundary having the maximum gradient, for example, is deflected downward as indicated by the line *d'd* and forms an image of the slit at the lower level *d'* on the diaphragm. Owing to the angle the diaphragm slit makes with the vertical, the portion of the light in the lower image *d'* that enters the slit is shifted laterally from the position at which the normal pencil enters by an amount proportional to the vertical deflection in the boundary gradient. The cylindrical lens consequently imparts to this pencil a corresponding lateral shift in the opposite direction to the position *d*, figure 4b, without affecting its vertical position, i.e., *d* of figure 4a.

The path of a pencil through another portion of the boundary is indicated by the line *e′-e* and forms the corresponding element in the pattern. All other elements in the complete pattern are formed similarly.

Our camera has been modified recently so that either the scanning or the cylindrical-lens procedures can be used interchangeably. The cylindrical lens has been mounted in the aluminum tube that serves as the camera "bellows" in such a manner that it may be easily placed in, or removed from, the path of the light forming the cell image. With this lens in the optical path, a mask with a diagonal slit is inserted at the schlieren diaphragm and the camera then yields patterns of the type shown in figure 3b. When, however, the scanning procedure is to be used, the diagonal slit is removed and the cylindrical lens moved out of the optical path. This arrangement is proving to be very satisfactory.

Although a slit as diaphragm has been described here in conjunction with the cylindrical lens, one may also use a diagonal straight edge, as was done by Philpot. Moreover, it is also possible to use either a slit (16) or a straight edge, in this case in a horizontal position, with the scanning method. With either method a slit as diaphragm gives the pattern as an illuminated line on a dark background, as is indicated in figure 3b, whereas in the case of a straight edge the pattern is obtained as the contour between a light and a dark field, figure 3a. The latter type of diaphragm has been criticized in that the position of the contour shifts slightly with the exposure and development of the photographic plate, whereas the center of the line obtained with a slit is more nearly independent of these factors. While this criticism of the straight edge is justified theoretically, no difficulty has been experienced in keeping the error from this source well within the limits of accidental error from other sources.

The cylindrical-lens method is convenient for visual observation during an experiment and for the control of electrophoretic separations, since the pattern may be viewed directly on the screen of the camera. For visual observation a slit diaphragm is preferable to a straight edge, since the greater quantity of light reaching the screen in the latter case causes a decrease in the apparent contrast between the pattern and the background. For photographic work, however, the straight edge is to be preferred, owing, in part, to the superior resolving power and simpler diffraction phenomena (3) characteristic of this diaphragm. Consequently the permanent photographic records of an experiment, on which the analyses are based, are obtained with a straight edge as diaphragm and with the scanning procedure. With the latter method the optical errors inherent in the uncorrected cylindrical lens are eliminated.

IV. THE ELECTROPHORESIS OF PROTEIN MIXTURES AND CHOICE OF THE BUFFER SOLVENT

Although the electrophoretic method may be used for precise measurements of the mobilities of pure proteins as functions of the pH and ionic strength of the buffer solution in which they are dissolved, the method is finding its widest application in the electrophoretic analysis of naturally occurring protein mixtures. Of the many mixtures that are available for study, the plasma proteins are among the most important. Consequently it is of interest to consider the sources of error in the electrophoretic analysis of plasma and to establish the experimental conditions, particularly the buffer solution used as solvent for the plasma proteins, that yield satisfactory patterns.

In an ideal electrophoresis of a protein mixture the volumes swept through by the rising and descending boundaries due to each component are identical and are proportional to the mobilities of the separate components. Moreover, the area under each "peak" in the electrophoretic pattern is proportional to the concentration of the component in the mixture to which it is due. In the ideal or limiting case the patterns for the two sides of the channel are mirror images of each other. The actual patterns, however, never exactly meet this condition and only approach it as the protein concentration is decreased. At such low protein concentrations the areas under the peaks are, however, not of sufficient magnitude to be determined accurately, and the vertical density gradients in the boundaries are frequently insufficient to stabilize the latter against the disturbing effects of convection.[3] The asymmetries between the patterns that appear as the protein concentration is increased are due to the contribution of the protein ions to the conductance and viscosity of the buffer solution in which they are dissolved and also to the influence of these ions upon the pH of the solution and upon the transference numbers of the buffer electrolytes. Typical

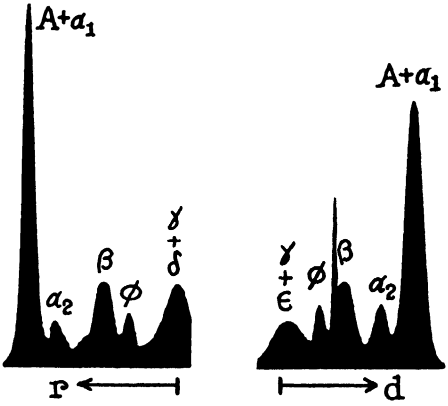

FIG. 5. Electrophoretic patterns of (r) the rising and (d) the descending boundaries of a human plasma, diluted 1:4, in a 0.025 *N* LiCl-0.025 *N* LiV-0.025 *N* HV (V = diethylbarbiturate) buffer at pH 7.9 after electrolysis, at 0.5°C., for 5750 sec. at 7.76 volts per centimeter. The initial boundary positions are indicated by the ends of the arrows.

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[3] Thus it has been found necessary to use a smaller current for the electrophoresis of, say, a 0.1 per cent solution of a protein in a given buffer than for a 1 per cent solution in the same solvent. The importance of the vertical density gradient in reducing disturbances due to convection and electroösmosis is also illustrated by the work of Shedlovsky and Smadel (14) on the elementary bodies of vaccinia.

asymmetries, i.e., "boundary anomalies," are illustrated in the patterns of figures 5 to 7. Thus the rising boundary due to albumin is sharper than the corresponding descending boundary. Boundaries characterized by large gradients, such as the rising albumin spike of figure 7, are not desirable for the practical reason that they cannot be recorded completely and, theoretically, because their areas are subject to the convergence and curvature errors discussed by Svensson (19). Moreover, all of the rising boundaries in the figures have swept through larger volumes, but have smaller areas, than the corresponding descending boundaries. Finally, there are the δ and ϵ peaks, of quite different areas, that do not correspond to any component of plasma. The ϵ boundary represents a gradient of buffer electrolyte concentration that remains, on electrophoresis, near the initial boundary position. The δ boundary, on the other hand, is due to concentration gradients of both buffer salts and proteins. Moreover, it was suggested in an earlier paper (7) that the salt gradients in the δ and ϵ boundaries are balanced, partially at least, by small changes of salt concentration across each of the protein boundaries. These inverted salt gradients in the protein boundaries decrease the area of the latter slightly and, as will be shown below, should be considered in interpreting the patterns.

TABLE 1

Buffer solutions used as solvents for the electrophoretic analysis of human plasmas and sera

(1) BUFFER	(2) μ	(3) pH AT 25°C.	(4) $\kappa_B \times 10^3$	(5) SERUM OR PLASMA	(6) DILUTION[*]	(7) NUMBER OF PEAKS	(8) SYMMETRY	(9) γ SEPARATION
0.1 N NaCac[†]-0.02 N HCac	0.1	6.8	3.10	P	1:2	[‡]	0.65	Yes
0.008 M NaH_2PO_4-0.064 M Na_2HPO_4	0.2	7.7	4.81	P	1:2	6[§]	0.39	Yes
0.004 M NaH_2PO_4-0.032 M Na_2HPO_4	0.1	7.7	2.62	S	1:4	5	0.52	Yes[¶]
0.025 N LiV[‖]-0.025 N HV-0.025 N LiCl	0.05	7.9	1.96	P	1:4	5	0.75	No
0.025 N LiV-0.025 N HV-0.075 N LiCl	0.1	7.9	3.97	S	1:4	4	0.75	No
0.025 N LiV-0.025 N HV-0.0675 N LiCl-0.0025 M $CaCl_2$	0.1	7.9	4.00	S	1:4	4	0.70	No
0.02 N NaV-0.02 N HV-0.08 N NaCl	0.1	7.9	5.13	P	1:2	5	0.54	No
0.04 N NaV-0.02 N HV-0.06 N NaCl	0.1	8.2	4.58	P	1:2	7	0.56	Yes[¶]
0.025 N $NaHCO_3$-0.1 N NaCl	0.125	8.2	6.64	S	1:4	4[§]	0.54	No
0.1 N NaV-0.02 N HV	0.1	8.6	3.03	P	1:2	7	0.81	Yes
0.1 N LiV-0.02 N HV	0.1	8.6	2.41	P	1:2	7	0.81	Yes[¶]
0.1 N NaOH-0.6 N glycine	0.1	9.0	3.48	P	1:2	6[§]	0.44	Yes[¶]

[*] With a dilution of 1:2 the new tall center section was used, otherwise the old short section.

[†] Cac = cacodylate.

[‡] Partial precipitation of the proteins occurred in this buffer solvent.

[§] Incomplete separation of α_1 from albumin.

[¶] Separation was less complete than in 0.1 N NaV at pH 8.6.

[‖] V = diethylbarbiturate.

The abnormally sharp spike usually observed in the descending β-globulin boundary is a different type of asymmetry from those mentioned above and appears to be characteristic of this component.

The author has obtained patterns of human plasma, or serum, in a series of buffer solutions the compositions, ionic strengths, pH values, and specific conductances of which are given in the first four columns, respectively, of table 1. Three of these patterns, all of the same sample of plasma, are reproduced in figures 5 to 7 and the corresponding experimental conditions are recorded in the legends for the figures. The patterns of figure 5 were obtained under the same conditions as in previous work (9) from this laboratory on plasma and serum. A comparison of the patterns of this figure with those of figure 6 indicates that in the 0.1 *N* sodium diethylbarbiturate buffer at pH 8.6, a previously unrecognized component, designated as α_1, has separated from the albumin. Also the δ and ϵ boundaries have separated from the peak due to γ-globulin, thereby permitting a more accurate determination of the concentration of this important component.

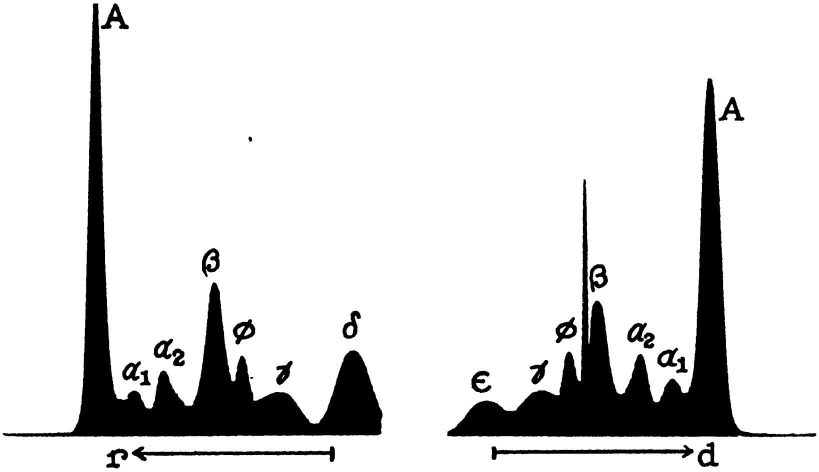

FIG. 6. Electrophoretic patterns of the same plasma as that shown in figure 5 but diluted 1:2 and dissolved in a 0.1 *N* NaV–0.02 *N* HV buffer at pH 8.6. Patterns were obtained after electrolysis for 14,000 sec. at 5.38 volts per centimeter.

The patterns of figure 7 were obtained in the buffer solvent used by Svensson (20) in his work on animal sera. In this solvent the α_1-globulin appears only as a shoulder on the albumin peak, fibrinogen, ϕ, is not adequately separated from the β-globulin, and the rising albumin boundary is too sharp to be recorded completely. Horse plasma was also examined in the same solvents as those used in obtaining the patterns of figures 6 and 7. In contrast with human plasma this material gave a more satisfactory pattern in the phosphate than in the diethylbarbiturate buffer, thereby suggesting that the proper solvent for the analysis of the plasma of a given type of animal varies with the species and should be determined experimentally.

Sufficient data pertaining to the patterns obtained in the other solvents listed in table 1 are included in that table to show that, on the basis of symmetry and number of peaks clearly resolved, none is superior to the 0.1 *N* sodium diethylbarbiturate solution at pH 8.6 used in obtaining the patterns of figure 6. The letter in the fifth column indicates whether serum, S, or plasma, P, was the material studied. The dilution factor is given in column 6 of the table and in the next column the number of clearly resolved peaks in the pattern. One more peak, due to fibrinogen, is expected in a plasma pattern than in a serum pattern. In column 8 is given the ratio of the maximum refractive index gradient in the descending albumin boundary to that in the rising albumin boundary. While this ratio depends upon a number of factors, it nevertheless furnishes an index

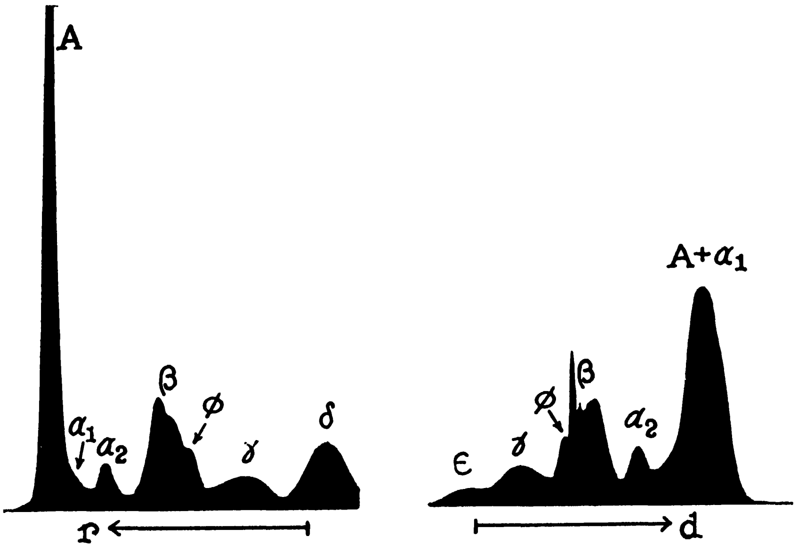

FIG. 7. Electrophoretic patterns of the same plasma as that shown in figure 5 but diluted 1:2 and dissolved in a 0.008 *M* NaH_2PO_4–0.064 *M* Na_2HPO_4 buffer at pH 7.7. Patterns were obtained after electrolysis for 20,500 sec. at 4.67 volts per centimeter.

of the symmetry of the patterns, the value approaching unity in the ideal case. As Svensson has suggested (20), a rapid spreading of the descending albumin boundary is undesirable, since the adjacent globulin peak is apt to be masked, as is the case with the α_1 component in figure 7d. Separation of the δ and ϵ boundaries from the γ-globulin peak is indicated in the last column of the table.

The same sample of material was not used in all of the experiments reported in table 1 and it is not possible, therefore, to make a quantitative study of the effect of the buffer solvent upon the relative concentrations of the components. There is some evidence (4, 15) that the buffer ions do have specific effects on protein mobilities. Consequently the redistribution of mobilities that occurs when one buffer solvent is substituted for another may cause appreciable variations in the apparent composition of a plasma. The following observation was, however, made in connection with the patterns represented by table 1: Of the solvents listed in that table, the 0.025 N sodium bicarbonate–0.1 N sodium chloride solution at pH 8.2 is nearest in ionic composition to that of blood, and the analysis of a serum in this solvent was in good agreement with the analysis of the same serum in the diethylbarbiturate solvent used in our early work on human plasmas and sera. Moreover, the patterns of figures 5 and 6 are of the same sample of plasma and yield closely agreeing results if account is taken of the fact that, in figure 5, α_1 migrated with the albumin and the γ-globulin did not separate from the δ and ϵ boundaries. These observations appear to justify the use of the diethylbarbiturate buffer as solvent for the plasma proteins, in spite of the fact that this buffer ion is not a normal component of blood.

Some additional observations in connection with table 1 are worthy of note. Although pattern asymmetries are reduced by increased ionic strengths, the improvement is generally more marked if the increase is due to buffer salt, which simultaneously raises the buffer capacity, than if it is due to neutral salt. A pattern obtained in a 0.1 N lithium diethylbarbiturate solution at pH 8.6 was quite indistinguishable from a pattern of the same material in a solution of the corresponding sodium salt, except that in the latter solvent greater separation between the δ, or ϵ, boundary and the γ-globulin boundary occurred. This observation has been of value in the interpretation of the δ and ϵ effects. In figure 6d, in which the end of the arrow indicates the initial boundary position, it can be seen that the separation between the γ and ϵ boundaries is due, in part, to a slight cathodic displacement of the latter boundary. This displacement is known to be proportional to the change of the transference number, T_+, with the concentration (10). From the conductance data of table 1 and the mobility of the sodium ion, one can estimate a value of $T_+ = 0.73$ for 0.1 N sodium diethylbarbiturate and 0.66 for 0.1 N lithium diethylbarbiturate. Since the change of transference number with concentration is roughly proportional to the deviation of T_+ from 0.5 (1), this change is less for the lithium salt than for the sodium salt and, in agreement with theory, the observed cathodic displacement of the ϵ boundary is less in the 0.1 N lithium diethylbarbiturate buffer than in the 0.1 N sodium diethylbarbiturate buffer solution.

V. INTERPRETATION OF THE ELECTROPHORETIC PATTERNS OF PLASMA

A. Determination of relative concentrations

Since the specific refractive increments of the electrophoretically separable plasma proteins have not yet been measured it is only possible, at present, to determine their concentrations as differences in refractive index and not in terms of protein nitrogen or dry weight. The available evidence indicates that the refractive index is proportional to the protein concentration as determined by other methods, although the proportionality factor doubtless varies with the nature of the protein. While it would be of considerable interest to compare concentrations, as determined from the areas of the electrophoretic patterns, with values given by other methods, the present lack of knowledge of the specific refractive increments cannot be considered a limitation of the electrophoretic method, since there is no *a priori* reason for selecting one method of measuring protein concentrations in preference to others.

In determining the area, A_i, due to a given component, i, in the pattern it has been customary to make a more or less arbitrary separation, owing to the fact that the gradients of the different boundaries overlap. Following the procedure of Tiselius and Kabat (23), this has been done by drawing an ordinate from the lowest point between two adjacent peaks. These ordinates are shown as dotted lines in figure 8, which is a tracing of the cathode pattern of figure 6. If it is divided in this manner, the areas under the various peaks have the values in arbitrary planimeter units, given in the first line of table 2. With the exception of the peak due to β-globulin, it is also possible to resolve, as described by Pedersen (17), the pattern into a series of symmetrical curves. These curves are indicated by the light lines of figure 8 and the corresponding areas are recorded in parentheses in table 2. The two methods yield values for the areas

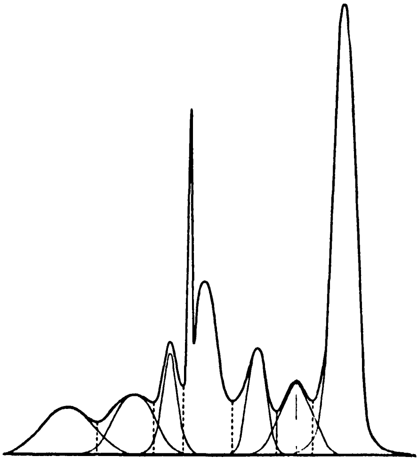

FIG. 8. Tracing of the electrophoretic pattern of figure 6d. In this diagram two methods of distributing the areas among the plasma components are illustrated.

TABLE 2

Concentrations and electrophoretic volume displacements of the components of a human plasma

	COMPONENT i	A	α_1	α_2	β	ϕ	γ	δ	ϵ
1	A_{id}	356 (343)	54 (72)	85 (64)	181 (199)	61 (50)	77 (76)		67 (77)
2	A_{ir}	325	45	75	163	56	77	141	
3	A_{id}/A_{Ad}		0.152	0.239	0.508	0.171	0.216		
4	A_{ir}/A_{Ar}		0.138	0.231	0.502	0.172	0.237		
5	V_{id} (ml.)	3.297	2.699	2.208	1.568	1.144	0.659		0.128
6	V_{ir} (ml.)	3.597	3.037	2.520	1.815	1.376	0.811	0.305	
7	ρ_i (eq. 5)	0.923	0.899	0.890	0.884	0.862	0.864		

that differ by as much as 33 per cent in the case of α_2-globulin and exhibit an average deviation of 15 per cent. The plasma globulins, with the exception of fibrinogen, are so far differentiated entirely on the basis of their electrophoretic mobilities. The differences in areas noted above are due, partially at least, to the fact that the two methods of dividing the pattern define the globulins by assigning to each a somewhat different range of mobilities. The method of Tiselius and Kabat enjoys the advantage of simplicity. In using the latter method the author has observed that the distribution of areas among the peaks shifts slightly as the distances between them increase and appears to approach that given by the Pedersen method. The arbitrary nature of the methods of separation must be recognized. The comparison made above may serve to indicate the uncertainties that arise from this source.

B. Assumptions

As previously noted, there are small salt gradients in each of the protein boundaries and the observed area, A_i, is thus the sum of an area, P_i, due to gradients of the i^{th} protein alone and an area, $-S_i$, due to salt, the negative sign indicating the inverted nature of this gradient. Since the magnitudes of these salt gradients are not known, except that they are relatively small, it has been found convenient to make certain assumptions regarding them in order to compute, from data obtained from the electrophoretic patterns, relative concentrations of the proteins. Thus the concentration of one protein component relative to that of another, say albumin, may be computed from values of A_i from the cathode pattern, designated as A_{id} since they refer to the descending boundaries, if the assumptions are made (I) that S_i is proportional to P_i and (II) that the proportionality factor is the same for all components. With these assumptions $P_{id}/P_{Ad} = A_{id}/A_{Ad}$. This relation was used in computing the concentrations of the plasma globulins, relative to albumin, from the pattern of figure 6; the results are given in the third line of table 2.

Values of A_i from the anode pattern, i.e., A_{ir}, since they refer to the rising boundaries, may also be used to compute relative concentrations if the additional assumption (III) is made that all of the protein components are held in the same proportion through the δ boundary. Values of A_{ir}/A_{Ar} are given in the fourth line of table 2 and are in reasonably good agreement with corresponding values of A_{id}/A_{Ad}.

The available evidence indicates that the first of the assumptions made in these computations, namely, that the salt gradients in a protein boundary are proportional to the gradients of protein, is valid.[4] The second assumption that the proportionality constant is the same for all proteins probably introduces, on the other hand, a small error, since it is unlikely that all proteins influence the transference of the buffer electrolytes to the same extent. The third assumption that all components are held in the same proportion through the δ boundary places restrictions, as will be shown below, upon the volume displacements of the various components as well as upon the areas. Since the displacements can be measured with somewhat more precision than the areas, it is thus possible to test the validity of the third assumption. In order to obtain the relations required for this test it is necessary to consider the transference of material from one side of the channel to the other during electrophoresis and to apply the principle of the conservation of matter to these processes.

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[4] The author has observed (see 13), for example, that the transference number of potassium chloride in aqueous solution varies linearly with the concentration of added glycine.

C. Electrophoretic transference of proteins and buffer electrolytes

Since the passage of an electric current does not produce changes of composition in the body of a homogeneous solution, the composition of the protein solution in the bottom section, and also that of the buffer solution in the two sides of the top section, remains constant during electrophoresis. The *total* area of an electrophoretic pattern is thus proportional to the integral

$$\int_B^P (\mathrm{d}n/\mathrm{d}h)\mathrm{d}h = n_P - n_B$$

in which n is the refractive index at the level h in the channel. The integration is from the original protein solution P to the buffer solution B and, since these limits are the same for both anode and cathode patterns, the total areas of the two patterns are the same and are independent of the time during electrophoresis at which the patterns are recorded. This relation may be written as

$$A_\epsilon + \Sigma A_{id} = A_\delta + \Sigma A_{ir}$$

or as its equivalent,

$$A_\epsilon + \Sigma(P_{id} - S_{id}) = P_\delta + S_\delta + \Sigma(P_{ir} - S_{ir}) \tag{1}$$

in which the summation is for all of the protein components and P_δ and S_δ are the portions of the area of the δ peak due to protein and salt gradients, respectively.

During electrophoresis the plasma proteins neither enter nor leave the cell. Consequently, if the cathode pattern indicates a loss of one of these components, the anode pattern should indicate a corresponding gain in that component. If V_{id} is the volume through which the i^{th} component descends, the loss of this component from the cathode channel is proportional to $V_{id}P_{id}$ and this must equal the corresponding gain, $P_{ir}V_{ir} - (P_{id} - P_{ir})V_\delta$, in the anode channel. Thus for each of the i components

$$V_{id}P_{id} = V_{ir}P_{ir} - (P_{id} - P_{ir})V_\delta \tag{2}$$

Considerable quantities of buffer electrolytes, on the other hand, move through the cell during an experiment. The total amounts of these materials in the cell at any instant remain essentially constant,[5] however, and from considerations similar to those above, i.e., conservation of matter, the following relation may be obtained:

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[5] Negligibly small quantities of buffer electrolytes may be gained, or lost, by the cell, owing to small volume changes resulting from electrophoretic separation if the specific volumes of the various components are not additive.

$$A_\epsilon V_\epsilon + \Sigma S_{id} V_{id} = S_\delta V_\delta + \Sigma S_{ir} V_{ir} \tag{3}$$

Since the net movement of buffer salts is from the anode to the cathode side of the channel and that of protein is in the opposite direction, the total transport of material is given by the difference between equation 2, summed over all protein components, and equation 3, i.e.,

$$\Sigma V_{id}(P_{id} - S_{id}) - A_\epsilon V_\epsilon = \Sigma V_{ir}(P_{ir} - S_{ir}) - V_\delta[S_\delta + \Sigma(P_{id} - P_{ir})]$$

but

$$P_i - S_i = A_i, \qquad \Sigma(P_{id} - P_{ir}) = P_\delta, \quad \text{and} \quad P_\delta + S_\delta = A_\delta$$

Therefore,

$$\Sigma V_{id} A_{id} - V_\epsilon A_\epsilon = \Sigma V_{ir} A_{ir} - V_\delta A_\delta \tag{4}$$

The relation 4 states that, if the area of each peak in the pattern be multiplied by the volume displacement of the peak, account being taken of the direction of migration, the sum of these products for the anode pattern is equal to the corresponding sum for the cathode pattern. Although equations 1 and 4 have been derived with reference to the electrophoretic analysis of plasma, they are valid for protein mixtures generally and represent two relations that may be used to advantage in checking these analyses. The relation 4 will be recognized as analogous to the well-known test for a successful transference experiment by the Hittorf or gravimetric method,—namely, that the loss of material at one electrode should equal the gain at the other electrode. From the data of table 2 a value of 1903 may be computed for the left-hand members of equation 4. This differs by 0.8 per cent from the value, 1887, obtained for the right-hand terms.

D. The δ boundary and a test of assumption III

Since P_{ir} and P_{id} are measures of the concentration of the i^{th} component on the two sides of the δ boundary, the ratio P_{ir}/P_{id}, which may be designated ρ_i, is the dilution that a component undergoes in this boundary. Solution of equation 2 for this ratio gives

$$P_{ir}/P_{id} = \rho_i = (V_{id} + V_\delta)/(V_{ir} + V_\delta) \tag{5}$$

an expression for ρ_i involving only boundary displacements. Values of the latter, as obtained from the pattern of figure 6d, are recorded in lines 5 and 6 of table 2, and in the last line of this table are reported values of ρ_i for each component as given by equation 5. It may be noted that the values of ρ_i are not strictly constant, as required by the assumption III that all components are held in the same proportion through the δ boundary, but show a drift that is outside the limit of error in the volume determinations.

It is also possible to compute values of ρ_i from determinations of area. Thus with the aid of assumption I, $\rho_i = A_{ir}/A_{id}$; i.e., ρ_i is the ratio of the area of the i^{th} peak in the anode pattern to that of the corresponding peak in the cathode pattern. In the case of plasma only the areas of the albumin peaks are of sufficient magnitude to give a reasonably accurate value, i.e., 0.913, for ρ_A. This value, it will be noted, is in satisfactory agreement with the corresponding value, 0.923, from the displacements of the albumin boundaries.

An average value, $\bar{\rho}$, for all components may be computed with the aid of equation 1, i.e.,

$$\bar{\rho} = \Sigma A_{ir}/\Sigma A_{id} = (A_t - A_\delta)/A_t - A_\epsilon)$$

in which A_t is the total area of either the anode or the cathode pattern. The data of table 2 yield the value of 0.909 for $\bar{\rho}$. This is also in approximate agreement with an average value of ρ_i from table 2. Thus in all cases in which the areas can be determined with sufficient precision they lead to values of ρ in essential agreement with those computed from the boundary displacements.

The small variations among the values of ρ for the different plasma proteins, as recorded in the last line of table 2, would appear to indicate that assumption III is not strictly valid but represents a close approximation. In the preceding discussion of the electrophoretic analysis of plasma, it has been tacitly assumed that there is no interaction between the protein components, i.e., that one protein has no influence upon the transference of another protein. From a comparison with systems in which interaction is known to occur (8), the plasma patterns appear to be relatively free from this effect, although it should be recognized that some of the observed asymmetries may possibly be due to such interaction.

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REFERENCES

(1) LONGSWORTH, L. G.: J. Am. Chem. Soc. **54,** 2741 (1932).
(2) LONGSWORTH, L. G.: J. Am. Chem. Soc. **61,** 529 (1939).
(3) LONGSWORTH, L. G.: Ann. N. Y. Acad. Sci. **39,** 187 (1939).
(4) LONGSWORTH, L. G.: Ann. N. Y. Acad. Sci. **41,** 267 (1941).
(5) LONGSWORTH, L. G., CANNAN, R. K., AND MACINNES, D. A.: J. Am. Chem. Soc. **62,** 2580 (1940).
(6) LONGSWORTH, L. G., AND MACINNES, D. A.: Chem. Rev. **24,** 271 (1939).
(7) LONGSWORTH, L. G., AND MACINNES, D. A.: J. Am. Chem. Soc. **62,** 705 (1940).
(8) LONGSWORTH, L. G., AND MACINNES, D. A.: J. Gen. Physiol. **25,** 507 (1941–42).
(9) LONGSWORTH, L. G., SHEDLOVSKY, T., AND MACINNES, D. A.: J. Exptl. Med. **70,** 399 (1939).
(10) MACINNES, D. A., AND LONGSWORTH, L. G.: Chem. Rev. **11,** 171 (1932).
(11) NEBLETTE, C. B.: *Photography, Principles and Practice,* pp. 38, 105. D. Van Nostrand Co., New York (1930).
(12) PHILPOT, J. S. L.: Nature **141,** 283 (1938).

(13) Roberts, R. M., and Kirkwood, J. G.: J. Am. Chem. Soc. **63,** 1373 (1941).
(14) Shedlovsky, T., and Smadel, J. E.: J. Exptl. Med. **72,** 511 (1940).
(15) Steinhardt, J.: Ann. N. Y. Acad. Sci. **41,** 287 (1941).
(16) Stern, K. G., and Du Bois, D.: Yale J. Biol. Med. **13,** 509 (1941).
(17) Svedberg, T., and Pedersen, K. O.: *The Ultracentrifuge*, p. 296. Oxford University Press, London (1940).
(18) Svensson, H.: Kolloid-Z. **87,** 181 (1939).
(19) Svensson, H.: Kolloid-Z. **90,** 141 (1940).
(20) Svensson, H.: J. Biol. Chem. **139,** 805 (1941).
(21) Tiselius, A.: Trans. Faraday Soc. **33,** 524 (1937).
(22) Tiselius, A.: Kolloid-Z. **85,** 129 (1938).
(23) Tiselius, A., and Kabat, E. A.: J. Exptl. Med. **69,** 119 (1939).

THE ULTRACENTRIFUGE

PRACTICAL ASPECTS OF THE ULTRACENTRIFUGAL ANALYSIS OF PROTEINS[1]

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The Svedberg equations for determining molecular weights by ultracentrifugal analysis are shown to be valid only if the proper significance is attached to certain of the involved terms. Emphasis is placed on the physical principles involved in the method, with special reference to the effects of hydration. Probable reasons are given for the formerly unsuccessful attempts to make accurate diffusion measurements in the ultracentrifuge, and a new method of increasing the precision of such measurements is presented. Various conditions of experimentation are discussed, and hydrostatic compression of the fluid being centrifuged is shown to exert no stabilizing action with respect to convective disturbances. Various types of ultracentrifuges are reviewed briefly. An explanation is presented of the working principles involved in recently developed optical methods based on the refraction of light.

I. INTRODUCTION

The history of ultracentrifugation dates back to the development of a small "optical centrifuge" by Svedberg and Nichols at the University of Wisconsin in 1923 (2). In the following year Svedberg and Rinde described improvements in the apparatus and its application to the determination of size-distribution among gold sols (3). They proposed the name "ultracentrifuge" for the new research tool and recommended that the term be reserved to denote an instrument by means of which sedimentation in a centrifugal field is measured quantitatively. They suggested that an ordinary laboratory centrifuge of high speed might be called a "supercentrifuge." However, in recent years it has become the practice of many investigators to associate the term "ultracentrifuge" with any type of high-speed centrifuge rather than with the original connotation of quantitative measurement.

A thorough bibliography of the literature pertaining to the construction and applications of ultracentrifuges of all types prior to 1940 has been included in *The Ultracentrifuge* by Svedberg, Pedersen, and their collaborators (1). The purpose of this paper is to review briefly from both a theoretical and a technical standpoint the principal points of the ultracentrifugal method, and to discuss some of its less generally recognized aspects which have been given attention in our laboratory.

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[1] Presented at the Symposium on Physicochemical Methods in Protein Chemistry, which was held under the joint auspices of the Division of Physical and Inorganic Chemistry and the Division of Biological Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September 8-12, 1941.

II. THEORY

Svedberg (12) has derived mathematical equations which show the relationship existing between the molecular weight of homogeneous particles and their sedimentation characteristics under certain conditions in an ultracentrifuge. These equations apply strictly only for dilute solutions of particles which are large in comparison with the molecules constituting the medium and which are electrically neutral with respect to the medium. Consider a suspension of particles of molecular weight M revolving in a centrifuge at an angular velocity of ω radians per second. Assume that the particles under observation are far enough from the bounding surfaces of the fluid to be uninfluenced by alterations of particle concentration in these regions. The net force acting on a mole of these particles at a distance x from the axis of rotation is simply the difference between their combined centrifugal weight and the buoyancy exerted by the displaced medium, i.e.,

$$F_c = M\omega^2 x - M\frac{\rho\omega^2 x}{\sigma} \quad \text{or} \quad F_c = M\omega^2 x - MV\rho\omega^2 x \tag{1}$$

where ρ is the density of the medium and σ is the density of the particles. The latter is equivalent to $1/V$, where V is the partial specific volume of the particles. Disregarding the negligible forces due to the slight acceleration in the speed of the particles with time, the force F_c is exactly counterbalanced by a frictional force F_s, which is proportional only to the sedimentation velocity of the particles and to a factor dependent upon the extent and shape of the surface of the particles. The frictional force on each mole per unit speed is called the frictional coefficient, f_s. Thus,

$$F_c = M\left(1 - \frac{\rho}{\sigma}\right)\omega^2 x = F_s = f_s\, \mathrm{d}x/\mathrm{d}t \tag{2}$$

where t is the time. In this expression $\frac{\mathrm{d}x}{\mathrm{d}t}/\omega^2 x$ is the sedimentation velocity per unit field of force and is usually denoted by s. Rewriting the equation, we have

$$s = \frac{M\left(1 - \frac{\rho}{\sigma}\right)}{f_s} \quad \text{or} \quad sf_s = M\left(1 - \frac{\rho}{\sigma}\right) \tag{3}$$

The diffusion constant D, which expresses the rate at which the particles will diffuse, is also inversely proportional to a molar frictional coefficient f_D:

$$D = \frac{RT}{f_D} \tag{4}$$

R is the gas constant and T the absolute temperature. It should be noted that a change in the mass of particles does not affect the diffusion rate directly but only in so far as it is associated with a change in the frictional coefficient. This is true by reason of the fact that the kinetic energy of a particle is independent of its mass. Theoretical and experimental evidence justify the assumptions that the random orientation of molecular particles during either diffusion or sedimentation is not appreciably altered and that, for the same medium at the same temperature T, $f_s = f_D$ to a very close approximation. Eliminating the frictional coefficient from equations 3 and 4, one obtains Svedberg's formula for the molecular weight as determined by the sedimentation velocity method:

$$M = \frac{RTs}{D\left(1 - \frac{\rho}{\sigma}\right)} \tag{5}$$

In general, sedimentation and diffusion experiments are performed at different temperatures and, for the sake of comparisons, it is customary to correct measured values of D and s to some standard temperature, usually $T = 293°$ Absolute. Svedberg has standardized conditions even further and expresses each measurement of sedimentation in terms of a "sedimentation constant," s_{20}, which is the rate the material would have in a hypothetical medium having the density and viscosity of water at 20°C. or 293° Absolute:

$$s_{20} = \frac{s\eta(\sigma - \rho_{w20})}{\eta_{w20}(\sigma - \rho)} \tag{6}$$

where η denotes viscosity.

If the particles under consideration are known to be spherical and unhydrated, the molecular weight can be determined without the diffusion constant by substituting in equation 2 the value of the molar frictional coefficient for spherical particles:

$$f_0 = 6\pi\eta N \left(\frac{3M}{4\pi N\sigma}\right)^{1/3} \tag{7}$$

in which N is the Avogadro constant. This method of estimating the size is often useful in the case of non-spherical molecules for which diffusion data are not available. It yields a minimum possible molecular weight regardless of the particle shape or the degree of hydration. When the Svedberg formula (equation 5) can be applied, some idea of the departure of the molecular species from the spherical shape can be obtained by comparing f_0 with f as determined from equation 2. f/f_0 is called the frictional ratio and always has a value equal to or greater than 1. However, values above unity may be caused by hydration, as well as by a lack of spherical symmetry, and experiments of another nature would be required to settle the question. Almost all the proteins studied by Svedberg (13) and his associates have shown frictional ratios between 1 and 2.

The sedimentation equations given above refer strictly only to particles isolated from the bounding surfaces of the fluid. However, Svedberg and Rinde (3) have shown that for the purposes of measurement use can be made of a small sector-shaped cell which has two flat walls oriented at right angles to the axis of rotation and two others which, if extended, would intersect along the axis. Particles originally close to any of these walls continue during sedimentation to pursue an average course parallel to the wall so that their radial movement proceeds just as if the bounding surfaces were further removed. However, with the passage of time the concentration of particles does progressively increase near the bottom of the cell and decrease at the top, but if the centrifugal force is sufficiently high, measurements of rate can still be made, as will be shown. The particles originally at the top or inner surface of the fluid migrate together, forming a "rear line of march" or moving boundary which demarcates the supernatant fluid and sedimenting solute. The sedimentation rate is determined by measuring the position of this boundary at intervals. Generally the boundary becomes progressively less well defined with time, because of a superimposed diffusion of particles across the boundary. However, the position which the boundary would have had in the absence of diffusion is simply the level at which the concentration is one-half that in the lower unaffected sections. When the rate of sedimentation is rapid enough in comparison to the diffusion process there exists for some time, between the diffuse boundary and the section of the cell in which the particles are accumulating against the wall, a region in which the concentration is nearly uniform throughout. The concentration in this region decreases at a steady rate during a constant-speed centrifugation because of diverging radial movements of the particles and because of the fact that the centrifugal force, and consequently the speed of migration, increases with the distance from the axis of rotation. The relation between this concentration C_t at any time t and the original concentration C_0 has been shown by Svedberg and Rinde (3) to be given by:

$$C_t = C_0\left(\frac{x_0}{x_t}\right)^2 \tag{8}$$

where x_0 and x_t are the respective radial distances of the meniscus and the boundary at time t.

If sedimentation is allowed to progress for a sufficiently long time, usually at a rotational speed considerably lower than that used for the sedimentation velocity method, a state of equilibrium is reached in which the tendency of the particles to sediment is exactly counterbalanced by their tendency to diffuse back into the medium. The molecular weight can then be determined by comparing the concentrations C_1 and C_2 of particles at two radial distances x_1 and x_2. The formula for the sedimentation equilibrium method as given by Svedberg is:

$$M = \frac{2RT \ln (C_2/C_1)}{\left(1 - \frac{\rho}{\sigma}\right)\omega^2(x_2^2 - x_1^2)} \tag{9}$$

The sedimentation equilibrium method is most useful for the investigation of purified preparations which are known to be homogeneous, since in a mixture the contributions of several species to the distribution of total concentration are not easily differentiated. The sedimentation velocity method, on the other hand, is ideal for the study of mixtures, since an individual boundary is obtained for each species the rate of which is sufficiently different from that of the other components. The method is of great assistance in judging the purity and homogeneity of a sedimentable material.

In the equations given for the determination of molecular weight it has been assumed, as already stated, that the particles are electrically neutral with respect to the medium, i.e., that they are not electrostatically attracted and influenced by smaller diffusible ions which sediment more slowly and diffuse faster. However, Tiselius (17) has shown that where electrical dissociation is present, the effect on the sedimentation rate of the principal particle can be rendered negligible by the addition of sufficient non-sedimenting electrolyte. About 1 per cent sodium chloride is usually used to suppress this Donnan effect.

III. HYDRATION

Of the several quantities which enter into the computation of molecular weight from sedimentation data, the density of the particles is the one which has aroused the greatest uncertainty, because it does not lend itself readily to direct measurement. The density of dried or crystallized material can be determined by measuring with a pycnometer the volume displaced by a known weight of substance. However, because of the possibility of hydration there is considerable doubt as to whether this represents the effective density of the particles sedimenting in a centrifugal field. This problem has been treated mathematically by Kraemer (15), who showed that even a comparatively large amount of hydration should not introduce a serious error into the determination of molecular weight according to the Svedberg equations. Because of the importance of hydration, it is considered worth while to discuss the physical principles which form the basis of the mathematical results. Usually the density of a suspending aqueous medium is not greatly different from that of water. As a simplification, consider a case in which they are equal. Even though the volume of a particle is then increased by the attachment or inclusion of water molecules, the net force tending to sediment the particle will not be altered, assuming that the average density of the fluid is not changed by the attachment. This is true by reason of the fact that the force acting on the bound water is exactly balanced by the buoyancy of the surrounding medium. It will thus be seen that the hydration has produced no alteration in equation 1, even though M is the molecular weight and σ the density of the unhydrated particle.

Although the net centrifugal force acting on the particle under consideration is unchanged by hydration, the size of the particle, and hence its frictional coefficient, is increased. Let the subscript h be used to differentiate quantities which refer to the hydrated state. If the same net force acts on the particle regardless of whether it is in the hydrated or unhydrated state, then, since $F_c = f\,dx/dt$, the sedimentation rates for the two cases will compare as the reciprocals of the respective frictional coefficients; i.e.,

$$\frac{s_h}{s} = \frac{f}{f_h} \quad \text{or} \quad sf = s_h f_h$$

This substitution for sf can be made into equation 3, giving

$$s_h f_h = M\left(1 - \frac{\rho}{\sigma}\right)$$

Equation 4 can, of course, be rewritten immediately for hydrated particles:

$$D_h = \frac{RT}{f_h}$$

Eliminating the frictional coefficient between these equations as before, we obtain for the molecular weight of the unhydrated molecule,

$$M = \frac{RTs_h}{D_h\left(1 - \frac{\rho}{\sigma}\right)} \tag{10}$$

which differs from equation 5 only in that the experimentally determined values of the diffusion and sedimentation rates have been obtained with hydrated particles. σ still refers to the unhydrated state. Thus the molecular weight of the unhydrated molecule is given by the Svedberg equation regardless of whether the corresponding sedimentation and diffusion measurements are made on material existing in a hydrated or unhydrated state.

Where the material bound from the medium by the particle is of a density different from that of the medium itself, we may proceed with the same argument, except that in starting, equation 2 is written as

$$F_c = \frac{M}{Z}\left(1 - \frac{\rho}{\sigma}\right)\omega^2 x$$

Here, Z is simply the ratio between the true weight of a mole and the true weight increased by the difference between the weight of the bound material and the weight of an equal volume of the suspending medium. The proportional error in a molecular weight determination is then $Z - 1$, which in most experimental cases is quite negligible.

Consideration of the principles outlined emphasizes the importance of performing the sedimentation and diffusion measurements in identical media under similar experimental conditions, in order that the respective frictional ratios will have the same value. Furthermore, it should be noted that the molecular weight determined by equation 10 is the molecular weight of particles in such a state that their density is the same as that which characterizes the material when the weight of a sample is determined. This is true whether the determination is based on chemical composition or on direct measurements with dried or crystalline material. It can be shown that the above considerations with respect to hydration and particle density apply in exactly the same manner when molecular-weight determinations are made by the sedimentation equilibrium method.

IV. APPARATUS

The Svedberg high-speed ultracentrifuge (5) rotor is driven by oil under pressure and rotates in a reduced atmosphere of hydrogen gas. The sector-shaped cells generally accommodate a column of solution about 16 mm. long and are spun in the rotor at speeds up to approximately 70,000 R.P.M. The thickness of the solution column parallel to the axis of rotation is usually less than 12 mm., and the width is only a few millimeters. The center of the cell is 65 mm. from the axis of rotation. The cell is provided with plane-parallel quartz windows which make it possible to direct light rays through the revolving solution in a direction parallel to the axis of rotation. Light passing through openings in a counterbalance or dummy cell at radial levels above or below the solution column is unaffected by the sedimentation and furnishes a convenient reference. Lower speed electrically driven centrifuges with smaller cells are used for making measurements of sedimentation equilibrium (1).

A simplified method of spinning rotors at high speed in a vacuum chamber by means of compressed air has been described by Pickels and Beams (6). Biscoe, Pickels, and Wyckoff (8) and also Bauer and Pickels (9) have incorporated this principle in the design of air-driven ultracentrifuges with rotor dimensions patterned after those of Svedberg. Alternative methods of spinning large rotors by compressed air at high speed have been described by Beams and Pickels (7) and by Pickels (10). More recently an electrical driving mechanism has been developed by Beams and Skarstrom (11).

V. OPTICAL METHODS

All the earlier sedimentation studies conducted by Svedberg and his collaborators were based on the light-absorption method first described by Svedberg and Rinde (3). Light of some suitable wave length which is absorbed by the sedimenting solute, but not appreciably by the solvent, is directed through the cell. Ultraviolet light from a mercury arc is usually employed for protein solutions. With visible light passing through a hemoglobin solution within the revolving cell, for example, the unaided eye sees a bright red annular band extending about the axis of rotation. The meniscus of the fluid appears as a fine line with the same center of curvature. The sedimenting boundary demarcating the clear supernatant and the solution is also concentric about the axis. To obtain a record of the sedimentation, it is only necessary to photograph at intervals a narrow cross-sectional strip of the total annular band of light. A camera of long focal length is used in order to avoid errors of parallax and to give good focusing for all depths in the cell. A typical sedimentation of a large homogeneous protein is illustrated by the series of photographs in figure 1. An absorption photograph showing two sedimenting components is shown in figure 2. These illustrations are discussed in more detail later in this paper.

Other optical methods which have to a great extent superseded the absorption method are based on the detection or measurement of deviations suffered by light rays passing through regions of refractive-index gradient such as exist at sedimenting boundaries. The method which can probably be made subject to the fewest optical errors is the refractive-scale method of Lamm (20).

Increasing attention, however, is being given to the considerably more convenient cylindrical-lens methods, the origin of which can be traced to the work of Thovert (19). Improvements and adaptations for the measurement of migrating boundaries have been described by Philpot, Svensson, and Andersson (21–23). Experience in our laboratory with a modification of Svensson's method has indicated that even with comparatively inexpensive optical parts a precision of measurement can be attained which is quite sufficient in view of the many minor uncertainties involved in the ultracentrifugal method. Special tests have shown that deviations of light produced at any level of the cell, at least deviations up to 0.02 radian, can be registered at the photographic plate with an error of less than 1 per cent, and that for all deviations in this range the image positions of every level in the cell can be produced with an accuracy of less than 1 per cent of the total cell height.

A schematic representation of the optical principles involved is given in the perspective drawing of figure 3. A camera lens is placed so as to form on a screen the images A′, B′, C′ of the three small holes A, B, and C in a thin diaphragm. A condensing lens is placed so as to focus the image of a horizontal line source on the front surface of the camera lens. Limit consideration to those light rays which pass through the small holes. It can be seen that the light eventually constituting each of the images A′, B′, C′ consists of rays which alternately diverge and converge in accordance with simple optical principles. Each set of rays may be thought of as making up a "light sheet" that varies in width but is always parallel to the horizontal line source. If the light sheet diverging from A toward the camera lens were deviated downward from the normal course at A, as indicated by the dotted lines, it would nevertheless converge at A′ by reason of the fact that the camera lens is focused to produce such a result. An analogy holds for all other images. Suppose that a cylindrical lens with vertical axis is added to the system in such a position that a point light source at the front surface of the camera lens would be focused as a vertical line image at the screen. The effect of this lens on each of the original light sheets will be to converge it

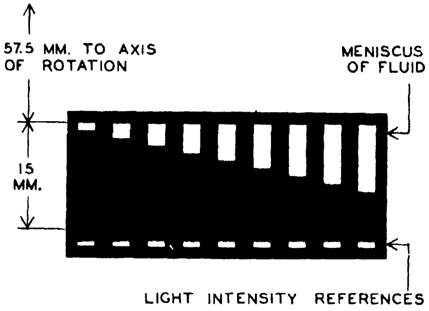

FIG. 1

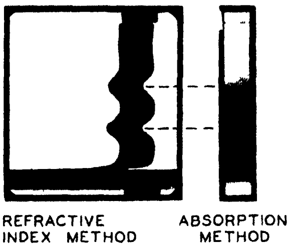

FIG. 2

FIG. 1. Sedimentation of monodisperse hemocyanin in the serum of *Limulus polyphemus*, as shown by the absorption method. Speed, 18,000 R.P.M.; interval between photographs, 15 min.; illumination, 3650 Å. line of mercury; cell thickness, 3 mm.; approximate concentration, 1.45 per cent.

FIG. 2. Sedimentation photographs illustrating correlation between absorption method and refractive-index method. Material, polydisperse hemocyanin in diluted, dialyzed serum of *Limulus polyphemus*; speed, 16,200 R.P.M.; time, 2.26 hr. after full speed; cell thickness, 1 cm.; total protein concentration, 0.41 per cent.

prematurely, so that it again diverges and forms a horizontal line image $A_1'A_2'A_3'$ on the screen instead of a point image. The vertical positions of the images are not altered by the cylindrical lens. A downward deviation (dotted lines) of the sheet at A would not alter the position of the line image. Suppose that over the front surface of the camera lens is placed a mask provided with a narrow inclined slit. It can be seen that of the undeviated rays through A, only the central one is now able to pass through the camera lens and reach the screen, where it will appear as a point of light A_2', as contrasted to the previous horizontal line image $A_1'A_2'A_3'$. Since the undeviated light sheets through A, B, and C all converge in a common image of the light source at the mask, it follows that B and C will also be represented at the screen, when there is no deviation at the diaphragm, by light points B_2' and C_2', which are situated along a central vertical line passing through A_2'. However, if the light sheet from A, for example, is deviated downward sufficiently from its normal course, then only the ray at one edge of the light sheet will pass through the camera lens, and the light point corresponding to A will appear on the screen at A_3'. The horizontal distance by which any light point will be displaced from the central line $C_2'A_2'$ is directly proportional to the downward displacement suffered by the corresponding light sheet at the diaphragm. The same holds true for light coming from B or from C, and thus the system is capable of translating a vertical deviation of light into a horizontal

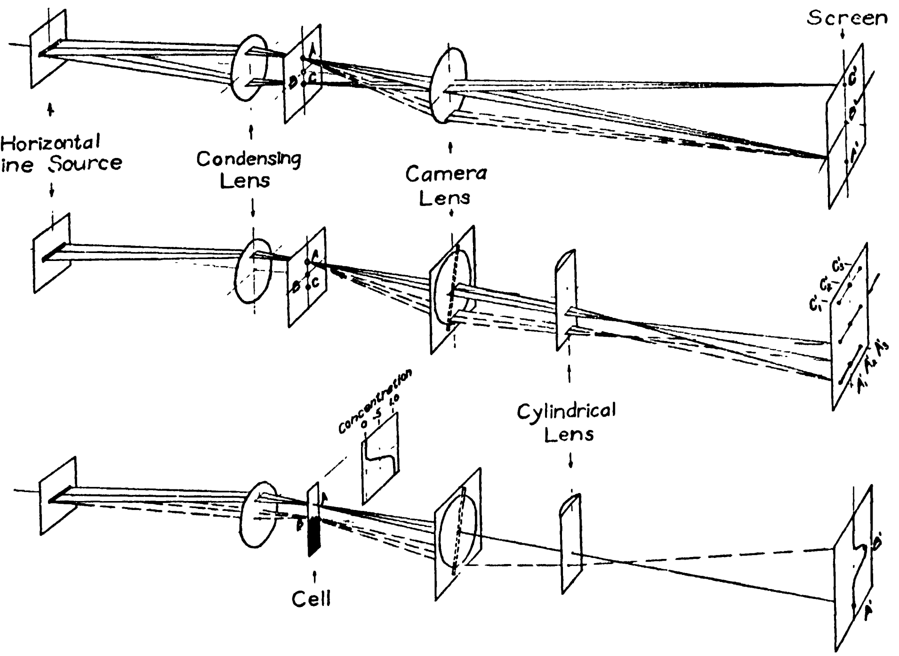

FIG. 3. Perspective schematic drawing illustrating the working principle of a refractive-index method employing a cylindrical lens.

displacement of a light point on the screen without altering the point's vertical height which is characteristic of a certain level in the cell.

Consideration will show that the action of the system is not altered by substituting narrow horizontal slits for the holes A, B, and C. For any given deviation of a light sheet, more rays will pass through the camera lens, but since they do pass through at the same point the cylindrical lens converges them into a point focus again at the screen. Now suppose that a cell containing an immobilized sedimentation boundary is substituted for the diaphragm in such a way that the maximum refractive-index gradient, i.e., the center of the boundary, is in the position B. The corresponding point B′ will be the one suffering the maximum horizontal displacement from the center line on the screen. Light coming through the cell below and above the boundary, as at A, is not deviated and the corresponding light points fall on the center line. Displacements of light points corresponding to successive positions within the boundary are proportional to the respective refractive-index gradients, with the result that the light points form a smooth continuous curve on the screen. The peak of the curve, if symmetrical, determines the position of the boundary; the area between the center line and the curve is proportional to the concentration of the material forming the boundary, assuming it is the only source of refractive gradients. With the ultracentrifuge there are extraneous gradients due to hydrostatic compression and distortion of the cell, and a curve incorporating these alone and called the base line must be obtained. If heavy salt ions are present in sufficient strength in the medium, they too produce gradients which must be taken into account by making separate ultracentrifugation runs with the medium alone (1). In applying the method to the centrifuge it is important to limit the light passing through the revolving cell to a zone having a width of not more than a few millimeters in a tangential direction.

In our laboratory the substitution of an opaque strip for the inclined open slit has produced certain advantages which will be described in a later paper. In place of a line of light as described above, a dark band against a bright background indicates deviations of light at various depths in the cell. Measurements are taken from either or preferably from both edges of this band.

The photographs of figure 4 illustrate an application of the refractive-index method employing a cylindrical lens. These were obtained during the same run as were those of figure 1. Figure 2 is an example of the simultaneous sedimentation of two protein components and shows the correlation existing between photographs taken of the same material at the same time by the refractive-index and the absorption methods.

For most cases the refractive-index method is preferable to the absorption method, particularly in the study of mixtures and where it is desired to determine the absolute or relative concentrations of several components. There is a considerably greater proportional variation in the absorption coefficients of proteins in the ultraviolet region, for example, than there is in their specific refractive indices in the visible region. As a matter of fact, good approximations of the concentrations of protein components of unknown optical properties can nearly always be made with the refractive-index method. It is also an advantage to be able to observe the sedimentation on the screen with visible light during sedimentation. Furthermore, it is more expensive and less convenient to work with ultraviolet light, which requires quartz optical parts.

A good refractive-index system is subject to fewer and less serious errors than is an absorption system. The pictures shown in figure 1 represent an unusually sharp boundary of a fairly concentrated, slowly diffusing material. Measurements can be made directly from the photographs. In actual practice, however, boundaries of smaller, more common proteins are more diffuse, such as those shown in figure 2. To analyze absorption photographs, it is then necessary to employ a microphotometer which will measure the photographic density of the plate at all levels in the cell picture. Results obtained must then be translated into measurements of concentration. The analysis is subject to all the usual photographic errors, as has been pointed out by Pedersen (1). The apparent position of a diffuse boundary and the apparent concentration distribution throughout the cell can be greatly altered by photographic overexposure or underexposure and also by the use of a highly absorbing preparation in the cell. It is imperative to use monochromatic light of high purity and to illuminate the cell uniformly.

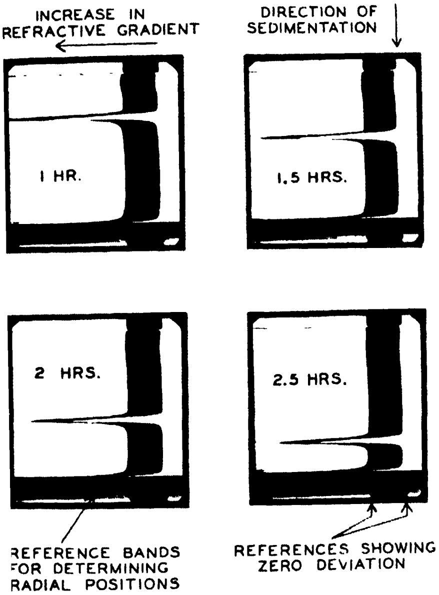
INCREASE IN REFRACTIVE GRADIENT
DIRECTION OF SEDIMENTATION
1 HR.
1.5 HRS.
2 HRS.
2.5 HRS.
REFERENCE BANDS FOR DETERMINING RADIAL POSITIONS
REFERENCES SHOWING ZERO DEVIATION

FIG. 4

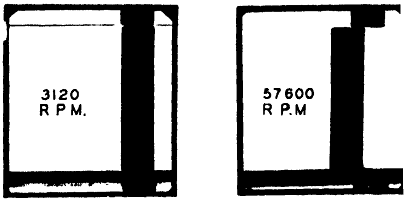

FIG. 5

FIG. 4. Pictures taken during the experiment illustrated in figure 1 and showing sedimentation in the same hemocyanin solution, as recorded by the refractive-index method.

FIG. 5. Photographs illustrating the gradients of refractive index introduced in a water-filled ultracentrifugal cell at high speed by hydrostatic compression and distortion of the ce

In some cases, however, the absorption method is to be preferred. For example, Pickels and Bauer (14) found that the virus of yellow fever in infected monkey serum showed a high differential absorption in the upper ultraviolet region, and they were able to study, by absorption, sedimentation boundaries which could not be detected with the refractive-index system. Since the precision of the refractive method depends on the magnitude of the concentration gradients present rather than on the concentration itself, the absorption method is sometimes more applicable with unusually ill defined boundaries or with very inhomogeneous preparations.

VI. EXPERIMENTAL CONSIDERATIONS

As Svedberg (13) has indicated, it is necessary that the sedimenting material be not disturbed by convection currents in the solution. In our work it has been found very important to have the cell aligned correctly within the rotor to prevent sedimentation of particles against the side walls. When even slight departures are made from this rule, photographs are obtained with a monodisperse substance which falsely give the impression of varying amounts of inhomogeneous material sedimenting below the main boundary.

The principal potential sources of convection currents are temperature gradients which must exist within the cell to insure the passage of heat from the sections of the rotor exposed to the greatest proportion of frictional resistance to those exposed to smaller amounts. The convection action is enormously magnified by high centrifugal force and can interfere seriously with the sedimentation of slowly moving particles. The gradients are quite small in most modern ultracentrifuges, having been estimated at about 5×10^{-4} °C. per centimeter for a rotor of the vacuum-type ultracentrifuge operating at 60,000 R.P.M. in rarified air at a pressure of 5×10^{-7} atm. However, gradients should not be discounted when the rotor is spinning under less favorable circumstances, for instance, in hydrogen gas at a pressure of 0.025 atm. Theoretically a density gradient due to temperature differences would then be of such a magnitude that a concentration gradient of protein amounting to 10^{-3} per cent per centimeter would be required to neutralize it. Wherever concentration gradients are very small, which is the case above and below boundaries of monodisperse proteins, for example, convection can take place, and it should be expected that the behavior of a diffuse boundary of a material in an unusually low concentration might easily be influenced.

It is a common impression that the radial density gradient introduced into a liquid by the application of centrifugal force is a marked stabilizing influence against convective disturbances. In a filled ultracentrifuge cell revolving at 57,600 R.P.M., for example, the outer layer of liquid is actually at least 1 per cent more dense than the inner layer. The magnitude of the concentration gradient is readily visualized by reference to the illustrations in figure 5. Only a small proportion of the refractive-index gradient is due to distortions of the cell. The maximum temperature gradient through the cell, for instance, could never cause a density gradient of the order cited, and it might at first thought seem that the compressed fluid would tend to remain in the lower part of the cell and thus prevent any stirring which might otherwise be caused through the action of weak opposing gradients due to thermal influences. The fallacy of this viewpoint can be realized by considering the energy changes in the system when it is disturbed while free from temperature gradients. If the state of the system is the most stable one, i.e., the one of lowest potential energy, then any interchange of fluid at different heights by some outside mechanism should increase the potential energy of the system with respect to the axis of rotation. Actually, if the top and bottom layers of fluid are interchanged, their respective densities reverse immediately in accordance with their new locations and the potential energy of the system is just the same as before. The result would be quite different if a cold (dense) layer of water at the bottom of a cell were exchanged with a warm (less dense) top layer. In this case the density of neither layer would be affected immediately by the transition, the potential energy would be increased, and the system would try to seek its original condition. The fundamental difference is that while the compressional density is a direct function of the depth below the fluid surface, the thermal density is not. Therefore, no account has to be taken of the compressional effects in considering the disturbances caused by temperature gradients in the ultracentrifuge.

It is, of course, important to know accurately the temperature of the solution while it is revolving in the ultracentrifuge in order to compare its viscosity with that of the standard medium. Adequate methods for making such measurements are in use with both the oil-driven (1) and the air-driven (9) machines. Runs are usually made at temperatures within a few degrees Centigrade of room temperature. For the investigation of proteins which exhibit excessive instability under such conditions, Rothen has surrounded his air-driven ultracentrifuge rotor with stationary refrigerated metal surfaces which permit him to maintain the material under study near 0°C. (16). Hydrogen at a reduced pressure of about 0.1 mm. of mercury furnishes the thermal conduction. For reasons of stability, as well as to avoid convective disturbances, it is often advisable to perform long diffusion experiments at about +4°C. In view of the theoretical importance of making sedimentation and diffusion measurements at the same temperature, Rothen's arrangement promises to widen the scope of studies suited to the Svedberg equation.

In the earlier experiments of Svedberg (4), determinations of the diffusion constant were attempted upon the material under study while it was sedimenting in the ultracentrifuge. An argument in favor of the procedure was that the environmental conditions were identical for both sedimentation and diffusion measurements. In later years it was found that the diffusion values obtained in this manner were not very reliable. The probable reason for the discrepancy between diffusion measurements made in the centrifuge and in a diffusion apparatus can be understood when one considers that a small inhomogeneity in the sedimentation rate of a group of particles can produce a comparatively large effect as far as the sharpness of a sedimentation boundary is concerned. For example, small amounts of aggregated or denatured material settling at slightly faster rates than the primary particles could produce such an effect. Small amounts of convection below or above the boundary could definitely change its form and apparent diffusion rate without appreciably altering the sedimentation rate. The potentialities of this mechanism must be considered when low concentrations of material are used. It will be recalled in this connection that the Svedberg equations apply strictly only for very dilute solutions in which no interaction between particles can be assumed. It has been the practice of Svedberg and his associates to make determinations of the sedimentation rate on a series of progressively lower concentrations of the solute under investigation. The results are extrapolated to zero concentration, and the respective sedimentation rate obtained is assigned as the true sedimentation constant of the material. Often there have been unexpectedly large differences between the rates for different dilutions (1). Most of the variations have been explained on the basis of a dissociation phenomenon. It is quite possible, however, that some of the discrepancies at very low dilutions were caused at least partly by convection.

Even with a convection-free, monodisperse system there is still another factor which affects the sharpness and apparent diffusion rate of a sedimenting boundary. Its influence increases with the concentration. There is through the boundary a relatively rapid shift in the solute concentration, so that the sedimenting particles at the trailing edge of the boundary are in a very dilute concentration compared to those on the leading edge. The more widely separated particles tend to sediment slightly faster, since the effective viscosity of the medium increases to some extent with increasing concentration of the sedimenting component, possibly because some interaction phenomenon exists between crowded particles, or perhaps because the effective buoyancy force is a function of concentration, or possibly because the backward streaming of the displaced medium becomes an important factor at higher concentrations. In any event, the boundary tends to resharpen itself continually in opposition to the tendency of the solute to diffuse. The effect is well illustrated by the photographs of figures 1 and 4. They show boundaries which with time spread only a small proportion of the amount to be expected from measured values of the diffusion constant. The contrast with the appearance of the more dilute boundaries in figure 2 is at once evident.

In our laboratory, tests are being made of a method by which it is hoped that reliable diffusion measurements can be made in the ultracentrifuge under certain conditions. It would offer great advantages in the study of mixtures the individual components of which cannot conveniently be isolated in pure form for diffusion measurements. With the vacuum-type ultracentrifuge it has been found possible to decelerate the rotor to very low speeds without disturbing boundaries partially sedimented rapidly at high speed. The boundaries are then allowed to diffuse, and diffusion constants are determined from measurements taken at the low speed. With insignificant centrifugal force, the migration of the boundary becomes negligible and the principal objections to the determination of diffusion constants in the ultracentrifuge cell are avoided. Minor corrections for the sectoral shape of the cell can be applied.

VII. CONCLUSION

The development of the ultracentrifugal method by Svedberg and his collaborators and their application of the technique to the investigation of molecular weights have become accepted as outstanding scientific achievements. The theoretical validity of the method is well established, and the principal sources of error in the earlier experimental procedures appear to have been realized and corrected. The field of research has become even more promising with time, and at present the scope of application is still being widened by further refinements and by the introduction of new related techniques.

REFERENCES

General

(1) SVEDBERG, T., AND PEDERSEN, K. O.: *The Ultracentrifuge.* Clarendon Press, Oxford (1940).

Oil-driven ultracentrifuge

(2) SVEDBERG, T., AND NICHOLS, J. B.: J. Am. Chem. Soc. **45,** 2910 (1923).
(3) SVEDBERG, T., AND RINDE, H.: J. Am. Chem. Soc. **46,** 2677 (1924).
(4) SVEDBERG, T., AND NICHOLS, J. B.: J. Am. Chem. Soc. **49,** 2920 (1927).
(5) BOESTAD, G., PEDERSEN, K. O., AND SVEDBERG, T.: Rev. Sci. Instruments **9,** 346 (1938).

Vacuum-type ultracentrifuge

(6) PICKELS, E. G., AND BEAMS, J. W.: Science **81,** 342 (1935).
(7) BEAMS, J. W., AND PICKELS, E. G.: Rev. Sci. Instruments **6,** 299 (1935).
(8) BISCOE, J., PICKELS, E. G., AND WYCKOFF, R. W. G.: J. Exptl. Med. **64,** 39 (1936).
(9) BAUER, J. H., AND PICKELS, E. G.: J. Exptl. Med. **65,** 565 (1937).
(10) PICKELS, E. G.: Rev. Sci. Instruments **9,** 358 (1938).
(11) BEAMS, J. W., AND SKARSTROM, C.: Rev. Sci. Instruments **11,** 398 (1940).

Theory and experiment

(12) SVEDBERG, T.: Chem. Rev. **20,** 81 (1937).
(13) SVEDBERG, T.: Proc. Roy. Soc. (London) **A170,** 40 (1939).
(14) PICKELS, E. G., AND BAUER, J. H.: J. Exptl. Med. **71,** 703 (1940).
(15) KRAEMER, E. O.: J. Franklin Inst. **229,** 393 (1940).
(16) ROTHEN, A.: Ann. N. Y. Acad. Sci., in press (1941).
(17) TISELIUS, A.: Z. physik. Chem. **124,** 449 (1926).

Optical methods

(18) TOEPLER, A.: Ann. Physik Chem. **131** ([V] 11) 33, 180 (1867).
(19) THOVERT, J.: Ann. phys. [9] **2,** 369 (1914).
(20) LAMM, O.: Nova Acta Regiae Soc. Sci. Upsaliensis [4] **10,** No. 6 (1937).
(21) PHILPOT, J. ST. L.: Nature **141,** 283 (1938).
(22) ANDERSSON, K. J. I.: Nature **143,** 720 (1939).
(23) SVENSSON, H.: Kolloid-Z. **87,** 181 (1939).

THE INVESTIGATION OF PROTEINS BY DIFFUSION MEASUREMENTS[1]

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It is the object of the present contribution to the Symposium to review the method of diffusion as a tool for investigating the properties of proteins in solution. While the diffusion process has been known to the physical chemist for a long time, its importance for biochemical research was recognized only more recently. Modern developments in the field of protein chemistry, in particular, have contributed to a revival of the interest in diffusion measurements which, together with sedimentation and electrophoretic measurements, constitute the most revealing methods for determining the state of dispersion of protein solutions. It is the aim of the present review to demonstrate the usefulness of diffusion measurements for a study of proteins, and to consider the theoretical and practical limitations of the methods available at present.

I. THEORY

It would take us too far afield to discuss here in detail the theory of diffusion. Reference may be made to the earlier publications on this subject, excellently reviewed by Williams and Cady in this journal (56), and to the more recent kinetic treatment of the diffusion problem by Eyring and coworkers (6, 11). In the following, we shall merely review briefly the elementary principles of the diffusion process as it is most frequently met in actual experimentation.

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[1] Presented at the Symposium on Physicochemical Methods in Protein Chemistry, which was held under the joint auspices of the Division of Physical and Inorganic Chemistry and the Division of Biological Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September 8-12, 1941.

We consider a system in which a solution of concentration c_0 is in contact with its solvent, as shown in the top section of figure 1. The boundary between solution and solvent is at the point $x = 0$, and positive values are assigned to x in the direction of increasing concentration. It will be assumed that no external force other than that of osmotic pressure is acting on the solute molecules and that the solute concentration is in the "ideal solution" range. Diffusion is confined to the vertical direction, while the force of gravity maintains the concentration constant within the horizontal plane. Under these conditions, the rate of diffusion is, according to Fick,

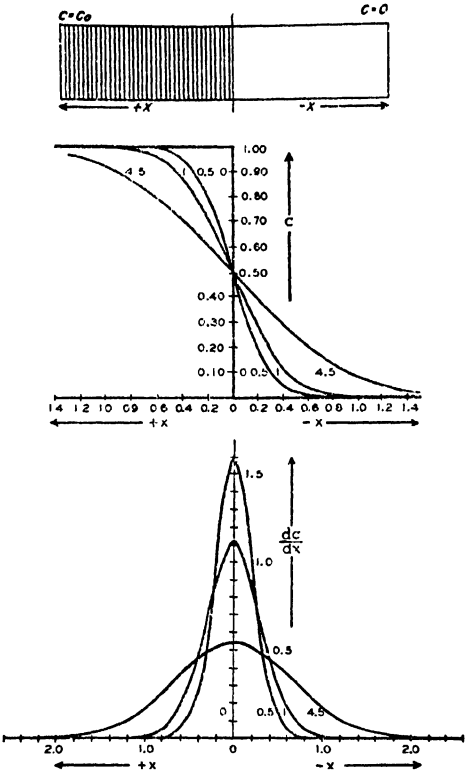

FIG. 1. Relation between concentration and distance of migration in a diffusion column. Top section: graphical illustration of the diffusion column. Center section: relation between concentration and distance of migration. Bottom section: relation between concentration gradient and distance of migration. The curves as drawn refer to diffusion times of 0.5, 1, and 4.5 hr., respectively. The whole diagram should be rotated counterclockwise about an angle of 90° in order to conform with the proper space directions.

$$\frac{ds}{dt} = -DA\frac{dc}{dx} \qquad (1)$$

where ds is the quantity of solute which in the time dt diffuses across a boundary of cross-sectional area A, under the influence of a concentration gradient dc/dx.

D is the diffusion constant, characteristic of the physical properties of the solute molecules in question. Its dimensions are square centimeters per second. Expressing mass in terms of concentrations, equation 1 may be reduced to the differential form

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{2}$$

The solution of this equation depends on the conditions imposed by the experimental methods applied for determining the diffusion rate.

For the conditions as stated above, the solution of equation 2 must fulfill the following boundary conditions: for $t = 0$,

$$c(x, 0) \begin{matrix} = 0, & \text{for } x < 0 \\ = c_0, & \text{for } x > 0 \end{matrix}$$

where c_0 is the initial solute concentration. Assuming that during the diffusion process no concentration changes occur at the extreme ends of the cell, integration of equation 2 leads to the following relation between c, x, and t:

$$c_x = \frac{c_0}{2}\left(1 - \frac{2}{\sqrt{\pi}}\int_0^y e^{-y^2}\,\mathrm{d}y\right) \tag{3}$$

where

$$y^2 = \frac{x^2}{4DT}$$

Diffusion constants, D, can be calculated from either the c–t relation at constant x, or from the c–x relation at constant t. Both methods are used in actual experimentation.

The relation between c and x, for various constant values of t, is shown in the center section of figure 1. It will be noted that at the position of the original boundary between solution and solvent, $x = 0$, c_x is equal to $c_0/2$ during the entire experiment and that the curves flatten out as t increases. The light-absorption method, discussed below, furnishes a relation of this type. If the differential, $\mathrm{d}c/\mathrm{d}x$, of the curves shown in the center section of figure 1 is plotted against x, a family of curves is obtained as shown in the bottom section of figure 1. In the ideal case, these curves have the shapes of Gaussian distribution curves and are identical with one another with respect to their areas. Curves of this type are obtained with the refractometric diffusion method. They follow the equation

$$\frac{\mathrm{d}c}{\mathrm{d}x} = \frac{c}{2\sqrt{\pi Dt}}\,e^{-\frac{x^2}{4Dt}} \tag{4}$$

II. Experimental Methods

While a large number of methods have been designed from time to time to measure the diffusion rates in solution, we shall confine this discussion to those methods which have found general application to proteins.

A. THE POROUS-DISK METHOD

The first method to be described is based on the diffusion across a porous diaphragm which separates solution from solvent. It was introduced by Northrop and Anson (36) and by McBain and Liu (22). The diffusion cell consists of a bell-shaped Pyrex-glass vessel, closed at the narrow top end by a stopcock and at the wide bottom end by a sealed-in sintered-glass disk, as shown in figure 2. The cell is filled with the solution the diffusion rate of which is to be measured, and is immersed in a vertical position in the solvent, just touching its surface. The whole apparatus is mounted in a constant-temperature bath. After preliminary manipulations, necessary for establishing a diffusion gradient within the pores of the diaphragm, diffusion is allowed to proceed from the solution into the solvent, density differences and slight convection currents tending to keep the concentration uniform within each compartment. That is to say, diffusion takes place only within the sintered-glass disk of thickness x, the concentration gradient dc/dx being constant within the pores, provided the diffusion rate is independent of solute concentration. Concentrations are determined after varying time intervals in aliquots of solution and solvent, and diffusion constants are calculated from the respective concentration changes by means of the equation

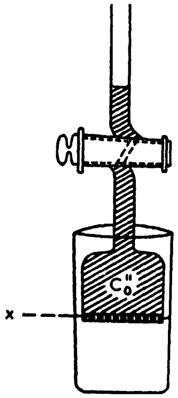

FIG. 2

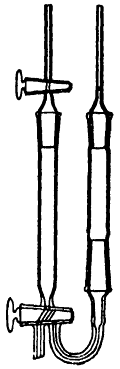

FIG. 3

FIG. 2. Diagram of the porous-disk diffusion cell (according to McBain and Liu (22)). x denotes the mean position of the boundary, c''_0 the initial solute concentration.

FIG. 3. Cylindrical diffusion cell, according to Svedberg (48) and Tiselius and Gross (53)

$$D_1 = \frac{1}{kt}\,\frac{V''V'}{V'' + V'}\left\{\ln c''_0 - \ln\left[c''_0 - \left(1 + \frac{V'}{V''}\right)c'\right]\right\} \tag{5}$$

where k is a constant, equal to the mean effective surface area of the disk, t the time of diffusion, c''_0 the initial concentration of the solution, c' the concentration of the diffusate, and V'' and V' the volumes of the solution and diffusate, respectively (23). D_1 is the integral diffusion constant representing a mean value over a range of varying concentrations. It is identical with the true diffusion constant D if the diffusion rate is independent of concentration. Otherwise, it can be related to it (23) by means of the equation

$$D = D_1 + c \frac{dD_1}{dc} \tag{6}$$

The constant k is determined by calibration with a solution of known diffusion constant, such as potassium chloride or sodium chloride. A thorough discussion of the method may be found in the papers by Mehl and Schmidt (23), Anson and Northrop (2), and McBain and Liu (22). The virtues of the porous-disk method are twofold. In the first place, it is simple experimentally and readily available. In the second place, it facilitates the determination of the diffusion constants of biologically active proteins, such as enzymes and viruses, in dilutions in which the concentration can hardly be measured by chemical methods but can be readily determined on the basis of biological activity. By comparing the rate of diffusion of the activity with that of a protein of known diffusion constant, the size of the smallest, biologically active, molecular kinetic unit can be estimated (10). The shortcomings of the method lie in the fact that it furnishes only a mean value for the diffusion constants if the solutions are polydisperse, and that only with the utmost care can satisfactory reproducibility of the results be obtained. The rather wide limits in experimental error that can be found in some of the published data (46) clearly emphasize this point. The initial decrease in diffusion rate observed by several investigators may represent a source of uncertainty worthy of further investigation. The method has been applied by Northrop and Anson (36) to hemoglobin, by Northrop to pepsin (35), by Scherp to trypsin[2] (45), by Kunitz and Northrop to chymotrypsinogen and chymotrypsin (14), by McBain, Dawson, and Barker to egg albumin (21), by Zeile (57) and by Stern (46) to catalase, and more recently by Hand to hemoglobin and catalase (10). A comparison of some of these results with those obtained by measurements of the absolute diffusion rate will be given in section III of this review.

B. THE LIGHT-ABSORPTION METHOD

The principle of this method is essentially the same as that employed in the earlier ultracentrifugal studies, and has been reviewed in detail by Svedberg and Pedersen (49). The method permits measurements of absolute diffusion rates and is based on the differences existing in the absorption spectra of solute and solvent. It may be described briefly as follows (53):

A transparent diffusion cell in which a sharp boundary between solution and solvent has been established is placed in a constant-temperature bath, between a light source and a photographic camera. Monochromatic light of such a wave length is used as will be absorbed by the solute but not by the solvent. With proteins containing no chromophoric groups, the absorption maximum lies in the ultraviolet region of about 270 mμ wave length. With others, such as hemoglobin or cytochrome, the absorption maximum is in the visible range of the spectrum. The cell is photographed at the beginning of the diffusion experiment and at certain time intervals thereafter, care being taken to keep the light intensity and exposure time constant. Under such conditions, the concentration distribution in the cell is imaged on the plate by a corresponding distribution of light intensity. The relation between concentration and degree of blackening of the photographic plate is obtained by filling the cell with known dilutions of the solution and taking exposures with the same light intensity and exposure time as were applied in the diffusion run proper. The reference plate and experimental plate are then measured with a microphotometer, yielding a relation between c and x of the type pictured in the center section of figure 1.

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[2] Scherp's value for the diffusion constant of trypsin is lower than that found for hemoglobin. As, however, the molecular weight of trypsin appears to be lower than that of hemoglobin, Scherp's data are probably erroneous.

The diffusion cell, as designed by Svedberg (48) and by Tiselius and Gross (53), is pictured in figure 3. It consists of a U-shaped glass tube, with the two limbs separated from each other by a three-way stopcock. The left limb is partly filled with the solution and the right limb with the solvent; a sharp boundary is formed by opening the stopcock after temperature equilibration in the constant-temperature bath. The boundary is then moved up in the solution compartment by means of a proper compensating arrangement until it reaches the center of the photographic field. The beginning of the diffusion process is made to coincide with the time of formation of the boundary.

Diffusion constants are calculated from the microphotometer registration curves by means of the equation

$$D = \frac{1}{4y^2}\frac{x_2^2 - x_1^2}{t_2 - t_1} \tag{7}$$

where x_1 and x_2 are distances from the boundary at the times t_1 and t_2, respectively. The most convenient method of calculation consists in determining from two successive curves (t_1 and t_2) the values of x which correspond to a given value of c, such as $c/4$ or $3c/4$. In that case, $1/4y^2$ is a constant. The method has been used by Tiselius and Gross (53) with protein concentrations as low as 0.2 per cent. The light-absorption method has given way in recent years to the light-refraction method, which with both sedimentation and diffusion measurements permits a considerably higher degree of accuracy.

C. OTHER METHODS

Before considering in detail the refractometric-scale method, mention will be made at this place of a diffusion method which, however, is more of theoretical significance than of practical value. It is due to Svedberg (48) and is based on the measurement of the degree of boundary blurring occurring during sedimentation in the ultracentrifuge. In sedimentation, the centrifugal force acting on the solute molecules is opposed by their diffusion. While, with such high centrifugal fields as are employed in sedimentation-velocity measurements, the rate of diffusion is small in comparison with that of sedimentation, nevertheless it becomes perceptible by a blurring of the originally sharp, sedimenting boundary.

The calculation of the diffusion constant is based on a comparison of the experimental sedimentation curves with those to be expected if no diffusion were to occur. Basic to this method is the assumption that the solutions under investigation are strictly monodisperse. The method is, however, rather inaccurate, owing to the fact that (*1*) the extent of diffusion taking place during a sedimentation run is actually very small, (*2*) the temperature in the ultracentrifuge is rarely sufficiently constant, and (*3*) any small inhomogeneity of the solution will have a significant influence on the apparent diffusion constant calculated from the sedimentation diagrams.

The method as applied to both the light-absorption and the light-refraction systems has been described in detail in Svedberg and Pedersen's monograph (49).

Bourdillon (3) recently designed a simple, yet rather crude, method for estimating the diffusion constant of viruses.

D. THE REFRACTOMETRIC-SCALE METHOD

This method is based on Stefan's (16, *loc. cit.*) phenomenon of the curvature of light when passing through a medium of varying refractive index. The method was introduced by Wiener (55) and Thoevert (51, 52) and further developed by Lamm (15, 16). The basic principle is illustrated in figure 4 which, for purposes of demonstration, has not been drawn to scale. O–O' is the optic axis, S the plane of a transparent scale, and C_1 and C_2 the vertical planes enclosing the diffusion cell; L_1 and L_2 are the principal planes of a photographic lens, and P is the plane of a photographic plate. We shall first assume that the medium contained in the diffusion cell has a constant index of refraction. In this case, light emerging from the point x_s on the scale will be deflected linearly between the planes C_1 and C_2 and will be imaged on the plate at x_1. If, however, there is a refractive-index gradient in the medium, increasing in extent in the downward direction, light emerging from x_s will be deflected in a curved fashion and will be imaged at x_2. The scale-line displacement, $x_1 - x_2$, is proportional to the differences between the exit angles of the undeviated and deviated light pencils (W_2 and W_1), which in turn is proportional to the refractive-index

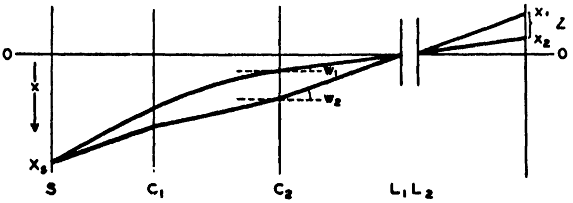

FIG. 4. Light path in the refractometric-scale method (not drawn to scale), according to Lamm (15, 16). O–O' is the optic axis, x the direction of increasing refractive-index gradient, S the plane of the transparent scale, C_1 and C_2 the planes enclosing the diffusion cell, L_1 and L_2 the principal planes of the camera lens, and P the plane of the photographic plate. W_1 and W_2 are the exit angles of the deviated and undeviated light pencils. For further explanations see text

gradient, dn/dx, existing in a horizontal plane at a depth conjugate to about the position of the point x_2:[3]

$$x_1 - x_2 = Z = Gl(W_2 - W_1) = Gab\,\frac{dn}{dx} \tag{8}$$

Here G is the photographic magnification factor, l the optical distance $\overline{P - L_1}$, a the optical distance $\overline{C_1 - C_2}$, and b the optical distance from the scale to the center of the cell[4]. By determining the scale-line displacement for every point on the scale, the refractive-index gradient distribution can be determined over the whole depth of the cell. This method yields, therefore, a relation between the refractive-index gradient and the distance from the boundary. Since, however, in general, the index of refraction of a protein solution is a linear function of its concentration, of the type

$$n_1 - n_0 = kc \tag{9}$$

where n_1 and n_0 are the refractive indices of solution and solvent, respectively, one virtually obtains a relation between concentration gradient, dc/dx, and x as shown in the bottom part of figure 1.

The diffusion assembly consists of a transparent diffusion cell placed in a constant-temperature bath, a transparent scale placed behind it, and a photographic camera placed in front of it, the camera lens being focussed on the scale. The scale is illuminated uniformly from behind by a suitable light source. The image of the undistorted scale lines is obtained by photographing the scale when the cell is filled with a medium of constant refractive index, usually the solvent (reference scale). The image of the displaced scale lines is obtained by taking exposures of the diffusion column at suitable time intervals after the beginning of the diffusion process (experimental scale). The scale-line distances are then measured on the plate in a microcomparator for both the reference scale and the experimental scale, and the differences in reading (equal to the scale-line displacements Z) are plotted as ordinates against the corresponding readings on the experimental scale as abscissae (equal to the positions of the displaced lines, z).

The experimental details of the method have been described in full by Lamm (16) and summarized in brief by Polson (42), Saum (44), and Neurath and Saum (33). In view of the general importance of Lamm's method and the present difficulties of obtaining access to some of the European journals, it may be appropriate to review here certain experimental details of a description of the apparatus which was built by the author several years ago and reconstructed since.

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[3] It is actually conjugate to the position of the point x_2 after the latter has been corrected for F (see equation 21).

[4] The optical distance is equal to the geometric distance divided by the refractive indices of the media traversed by the light.

1. *Apparatus*

An illustration of the diffusion assembly is given in figure 5. It is mounted on two horizontal I beams, bolted together in parallel position and resting on three concrete blocks. The light source (enclosed by a metal housing), light filters, and a simple condensing lens are mounted on an optic bench behind the constant-temperature bath. The light source is a Westinghouse high-intensity mercury lamp, H_3, 85 watts. Monochromatic light of 546 mμ wave length is obtained by a combination of Corning glass filters No. 551 and No. 512 and a Wratten filter No. 74.

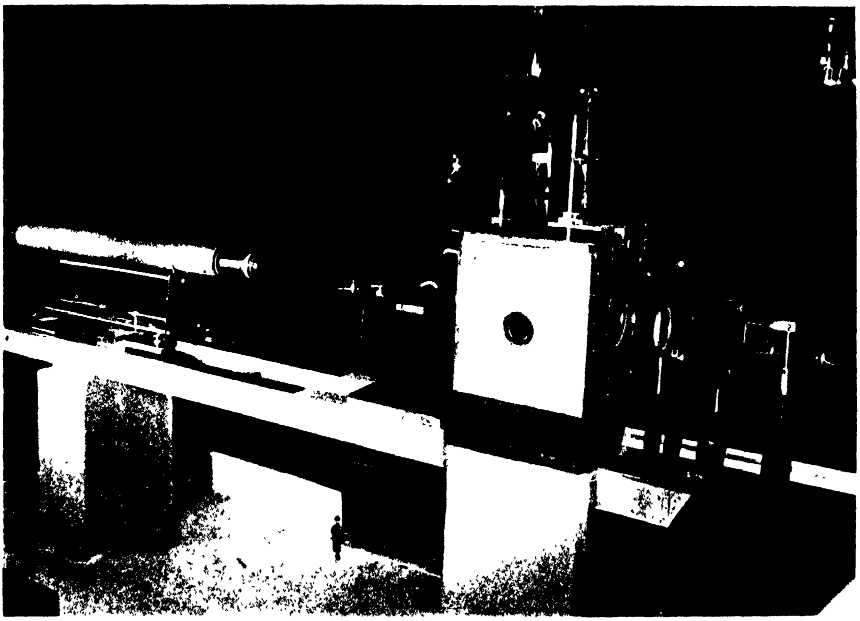

FIG. 5. Diffusion assembly for the refractometric-scale method, as used in the author's laboratory (for description see text).

A close-up view of the constant-temperature bath is shown in figure 6. The tank, 13 in. long in the direction of the optic axis, 16 in. wide, and 18 in. high, is made of brass and insulated by ¼-in. Celotex boards. Two circular glass windows, 4½ in. in diameter and ¼ in. thick, and optically flat to within 0.5 wave length, are mounted on the walls perpendicular to the optic axis while a third, smaller window is mounted on the wall facing the observer. This latter window serves merely for the observation of the cell during an experiment. The tank is cushioned against mechanical vibrations by several alternate layers of sponge rubber and Celotex and is isolated from the optical assembly, since the I beams of the latter pass through a channel in the supporting concrete block without any direct contact with the tank.

A cross-piece, running parallel to the optic axis and mounted on the top edge of the tank, holds the scale and the diffusion cell; the latter is attached to a movable rider which provides for variations in the cell scale distance. This distance is measured on a graduated metal scale mounted on the cross-piece.

The transparent scale is ruled on glass and protected by a cover glass, held in place by transparent Lucite cement. It is 5 cm. in length and graduated in 0.02-cm. divisions[5].

A 1/16th H. P., vertical-shaft motor stirrer is mounted separately on a floor flange. Splashing is avoided by a hollow metal cylinder surrounding the shaft and suspended vertically from the top cover of the tank. The cylinder is 3 in. in diameter and terminates about 3 in. above the stirrer blades. A mercury thermoregulator with stainless-steel housing, in conjunction with a thyratron vacuum-tube relay, keeps the temperature constant to within a few thousandths of 1°C.

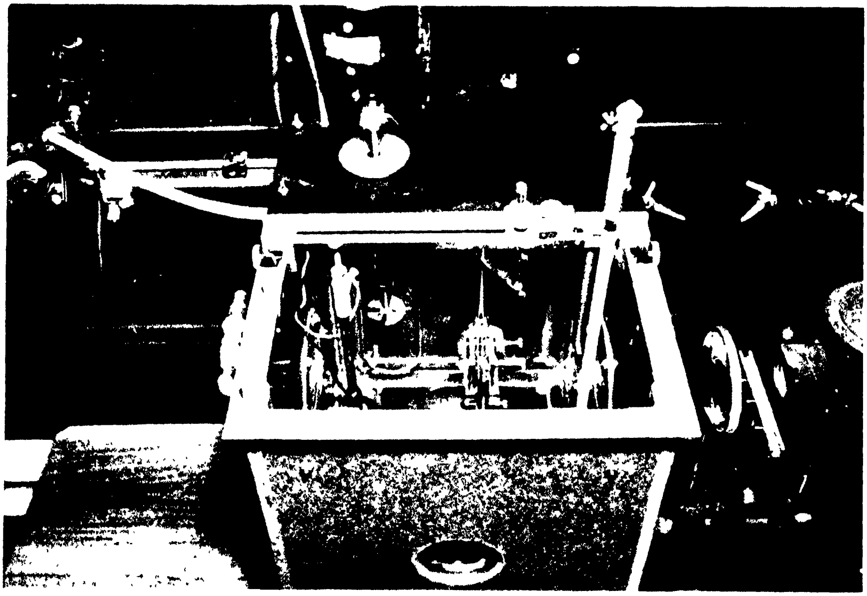

FIG. 6. Close-up view of constant-temperature bath of the diffusion assembly. The objects to be seen inside the tank are, from left to right, the thermoregulator, the stirrer with surrounding hollow cylinder, the cell, the scale, and the thermometer (for further description see text).

The camera lens, $1\frac{3}{8}$ in. in diameter, is a two-element projector lens of 24-in. focal length, corrected for spherical and chromatic aberration. It is focussed on the scale with a magnification of 1.1:1. A simple compur shutter provides for adjustment of the aperture (between f:50 and f:100) and exposure time. A slit in the back end of the camera and a movable plate-holder carriage allow a series of eight exposures to be taken on one plate. Eastman spectroscopic plates GV, sensitized for the green, proved to be best suited for sharp contrast and high light sensitivity. With an aperture of *f*:85, the exposure time is about 1 to 2 sec. The optical distances involved in the present diffusion assembly are as follows: plate to camera lens, 140.0 cm.; lens to scale, 113.9 cm.; *b*, the optical distance between the center of the cell and the scale, is variable between the limits of 3 and 11 cm.

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[5] Scales of this type may be obtained from the Gaertner Scientific Corporation.

The cell

In earlier studies the glass cell shown in figure 3 was employed. Its main disadvantage lies in the optical distortions produced by the lens effect of the cylindrical glass tube.

Lamm (16) designed a stainless-steel cell with plane-parallel windows, which eliminates most of the shortcomings of the Svedberg cell. A drawing of this cell is shown in figure 7. The cell consists of a stainless-steel disk with a rectangular, vertical slot in the center. Two optically flat, circular glass windows are pressed against the faces of the disk by threaded metal rings. A diaphragm, sliding in the horizontal slot and operated from the outside by a screw arrangement, serves to divide the vertical slot into two compartments.[6] The circular opening is filled with mercury through the right-hand side tube, in order to prevent leakage. The cell is filled with the solution to above the middle, with the diaphragm partly recessed. The lower half is then separated by advancing the diaphragm; the solution remaining above is rinsed out with solvent; and the top half of the cell is filled with the solvent. The boundary is then formed by slowly withdrawing the diaphragm. It is, of course, imperative that after each step of the above procedure the cell be brought to temperature equilibrium in the constant temperature bath in order to eliminate thermal convection and volume changes.

Recently, the author (27) has described a diffusion cell[7] which is somewhat similar to that previously introduced by Loughborough and Stamm (19). This cell operates on the principle of the Tiselius electrophoresis cell; however, unlike the conditions with the latter, the boundary is formed directly in the photographic field and thus does not have to be moved by special compensating arrangements. The advantage of this cell over the Lamm cell lies in the fact that solution and solvent surfaces are in direct contact as the boundary is formed, thus eliminating the displacement of the upper column of liquid occurring in the Lamm cell when the diaphragm is withdrawn. The latest model of the cell, a detailed description of which has already been published (27), is pictured in figure 8.

The cell consists of two U-shaped stainless-steel blocks which can be slid horizontally past each other by a screw arrangement. Two optically flat, rectangular glass windows are pressed against the vertical faces by the top and bottom clamps. The lower compartment, when separated from the top one, is filled with the solution through the left-hand tube, while the upper compartment is filled with the solvent through the right-hand tube. After temperature equilibration, the sharp boundary is formed by bringing the two compartments into vertical alignment. The dimensions of the cell proper are as follows: 1.7 cm. in the direction of the optic axis, 0.5 cm. wide, and 5 cm. high.

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[6] With the cell used in the author's laboratory, the stainless-steel diaphragm originally in use has been replaced by one made of polystyrene, in order to avoid scratching of the glass windows by the hard steel edges. Lubriseal has been found to be a very satisfactory sealing material.

[7] The cell was designed in cooperation with Mr. H. S. Bush, Instrument Maker of the Department of Chemistry of Cornell University, from whom it can be obtained.

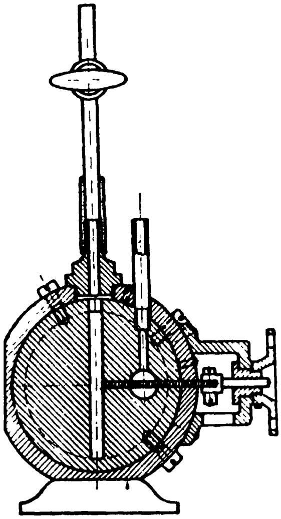

FIG. 7

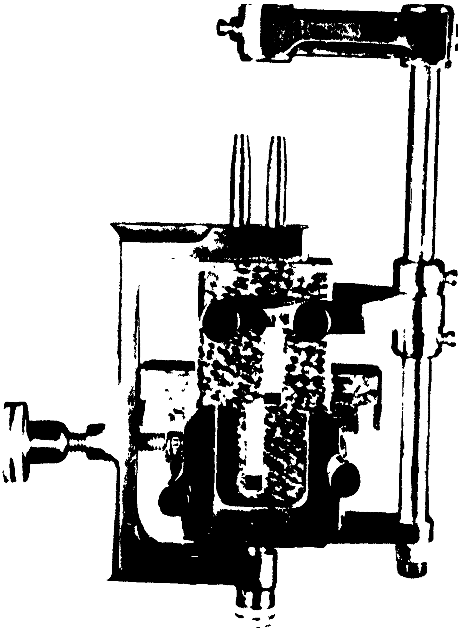

FIG. 8

FIG. 7. Schematic drawing of the Lamm diffusion cell (taken from reference 16)
FIG. 8. Photograph of the Neurath (27) diffusion cell (for description see text)

Lamm (16) has described a rectangular glass cell with a sliding glass diaphragm; however, no details of construction have been given.

Longsworth (18) and Rothen (13) have used the Tiselius electrophoresis apparatus for diffusion measurements. The diffusion curves were recorded by the schlieren-scanning method and the Philpot–Svensson (50) optical system, respectively. The results obtained in these studies will be referred to in section III of this review.

The earliest measurements carried out with the aid of a cylindrical lens and an inclined slit are those of Thoevert (52). It appears that they have not received the recognition they deserve; they are, therefore, reproduced in figure 9.

In figure 10 diffusion curves, as obtained by Longsworth[8] with the schlieren-scanning method, are reproduced.

2. *Measurements*

In order to measure the true diffusion rate of a solute, it is necessary to eliminate the influence of any external force other than that of osmotic pressure produced by the solute molecules in question. For this reason, the concentration gradient has to be negligible for all other ions and molecules that may be present; and, with solute molecules carrying electrical charges, such as the proteins, diffusion potentials have to be suppressed. Both requirements are met by carrying out the diffusion measurements in the presence of salts and by equilibrating the solution against the solvent, prior to the measurements, by dialysis through collodion or cellophane membranes. Salt concentrations of 0.1 ionic strength usually suffice to suppress diffusion potentials.

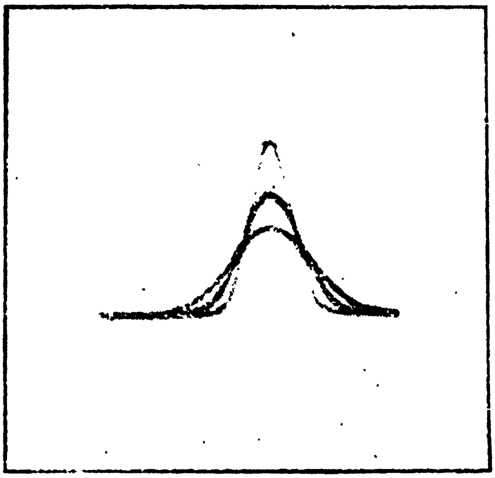

FIG. 9

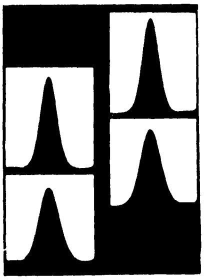

FIG. 10

FIG. 9. Refractive index gradient curves for the diffusion of sodium chloride, as obtained by Thovert (51) with the use of a cylindrical lens and an inclined slit. Reproduced from the original paper (52).

FIG. 10. Refractive-index gradient curves for the diffusion of a 1.4 per cent egg albumin solution, as obtained by the Longsworth schlieren-scanning method. The left- and right-hand pictures correspond, respectively, to the two boundaries in the Tiselius electrophoresis cell. The time of diffusion was 151,100 sec. in the top photographs and 235,800 in the bottom photographs. Unpublished experiments by Dr. L. G. Longsworth.

The diffusion cell is filled with solution and solvent as described above, and the time of diffusion is measured from the beginning of formation of the boundary. The length of the diffusion run, the time of the first exposure, and the time intervals between successive exposures depend on the diffusion rate of the protein under investigation and on the magnitude of the refractive-index gradients in the diffusion column.

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[8] The author is indebted to Dr. L. G. Longsworth for placing these photographs at his disposal.

Limitations on the over-all time of diffusion are imposed by both theoretical and practical considerations. The former have to do with the primary assumption involved in the derivation of equation 3, i.e., that the time of diffusion is short enough to avoid the occurrence of concentration changes at the extreme ends of the cell; the latter relates to the stability of the protein under investigation and requires that no chemical or physical changes occur during the diffusion process. Both factors tend to place an upper limit on the duration of the experiment.

The influence of the magnitude of the refractive-index gradient on the diffusion measurements can be best appreciated by considering certain approximations made in the application of the theory of the curvature of light to the present method (16).

The magnitude of Z, the scale-line displacement, depends on G, the optical magnification, b, the optical distance from the scale to the center of the cell, and ϑ, the angle of deviation of a light ray passing through the optically inhomogeneous medium contained in the diffusion cell (equation 8). The approximations involved in the derivation of this equation are as follows: (*1*) ϑ is sufficiently small to warrant the substitution of the arguments of the angles of inclination of the deviated light pencils with the optic axis for their tangents. Lamm has shown that for angles of 1.7°, this approximation introduces an error of 0.0003 radian. (*2*) n and dn/dx are constant in the region of the cell traversed by the light ray. This means, physically, that the vertical thickness of the light pencil is so small that the displacement Z is characteristic of a well-defined height in the diffusion column. Both conditions are more nearly fulfilled the longer the optical distance between camera lens and scale, the smaller the aperture of the lens, and the shorter the distance between cell and scale. A small aperture is also required on account of the lens action of the refractive-index gradient itself; however, a lower limit is placed on the aperture by the diffraction phenomena occurring when the aperture becomes too small. Values ranging between *f*:50 and *f*:80 are satisfactory. As a rule, b and the aperture will be made smaller for higher refractive gradients and larger for smaller gradients. From an extensive study of the influence of these various factors, Lamm (16) arrived at the conclusion that the theoretical requirements are fulfilled as long as the scale lines appear on the photographic plate undistorted and in focus, and this working rule can conveniently be used as a guide for actual experimentation. While too large differences in initial concentration must, therefore, be avoided, disturbances may also occur when the solute concentration is too low. In the latter case, the density difference between solution and solvent may become too small to keep the concentration gradients constant within the horizontal planes. The extreme limits in initial differences in concentration between solution and solvent lie in the neighborhood of 1.5 and 0.2 per cent protein, respectively.

After completion of the diffusion run the cell is filled with the solvent and another exposure taken, yielding an image of the reference scale. The scale-line distances are then measured in a microcomparator, with an accuracy of $\pm 1\mu$. The most convenient procedure consists in recording the comparator readings as determined from the same starting point on the microcomparator and setting the cross-hair on the same starting line on each scale image. The differences between the readings on the reference scale and experimental scale are then plotted as ordinates, against the respective comparator readings on the experimental scale as abscissae, thus yielding a curve which relates the scale-line displacements, Z, to the positions of the displaced lines, z.

3. *Methods of calculation*

(a) Monodisperse systems

The curves obtained in the manner described above are, except for a constant factor depending on the photographic enlargement and the optical cell scale distance, of the same form as equation 1. The calculations of the diffusion constant are based on the assumption that in the ideal case the curves have the properties of Gaussian distribution curves. We shall consider first the case of monodisperse solutions and ideal diffusion behavior. The following symbols will be used in the following equations which are most commonly used for calculating diffusion constants. Their meaning is also illustrated in figure 11.

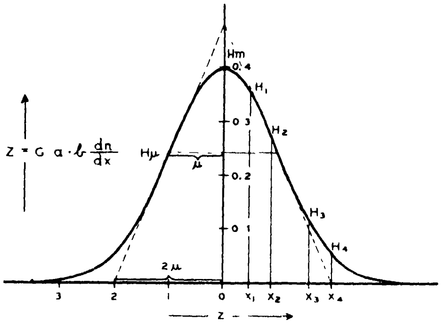

FIG. 11. Ideal scale-line displacement distribution curve (in normal coordinates). Z is the scale-line displacement, z the position of the displaced lines, H_m the maximum ordinate, μ half the distance between the inflection points, and H and x coördinates for points on the curve.

H_m = maximum ordinate,
μ = half the distance between the inflection points,
x_i = abscissa of the point i,
H_i = ordinate of the point i,
A = area under the curve,
σ = standard deviation,
t = time of diffusion, and
D = diffusion constant.

(*1*) *Maximum ordinate method.* The maximum ordinate divided by $\sqrt{e}$ gives the ordinate of the inflection points, H_μ. The diffusion constant is

$$D = \frac{\mu^2}{2t}, \quad \text{where} \quad H_\mu = \frac{H_m}{\sqrt{e}} \tag{10}$$

(*2*) *Method of successive analysis.* Here the diffusion curve is divided into a series of vertical and horizontal chords, and the diffusion constant is calculated from a pair of successive values of x_i and H_i.

$$D = \frac{x_2^2 - x_1^2}{4t \ln \frac{H_1}{H_2}} \tag{11}$$

In practice, the diffusion curve is divided on either side of the bisecting ordinate into about three to four ordinates equally spaced from one another, and the diffusion constants are calculated as described. For a monodisperse solution, the D values should agree with one another to within 2 per cent or better.

(*3*) *Maximum ordinate-area method.* If, in equation 4, x is taken as zero, then

$$\left(\frac{dn}{dx}\right)_{x=0} = H_m = \frac{n_1 - n_0}{2\sqrt{\pi D t}}$$

and (12)

$$D = \frac{(n_1 - n_0)^2}{4\pi t (H_m)^2} = \frac{A^2}{4\pi t (H_m)^2}$$

The area A is determined by graphical integration.

(*4*) *Statistical method.* This method, developed by Pearson (39) and applied to the present problem by Lamm (16), consists in treating the experimental curves as ideal displacement distribution curves. According to statistics, the standard deviation, σ, is equal to

$$\sigma = \sqrt{\mu_2^0} \tag{13}$$

where μ_2^0 is the second moment of the curve about the centroidal[9] ordinate· The diffusion constant D is related to σ by

$$D = \frac{\sigma^2}{2t} \tag{14}$$

In practice, the base line of the diffusion curve is divided into evenly spaced units of the breadth unity, numbered outward from an arbitrarily chosen origin near the center of the base line. If s_i is the respective number on the base line and S_i the corresponding ordinate, then the zero moment of the curve about the arbitrarily chosen central ordinate is

$$\mu_0' = \Sigma(S_i) = N \tag{15}$$

---

[9] The centroidal ordinate is that ordinate about which the first moment is equal to zero.

and is equal to area in the S units that were chosen μ_1', the first moment about this ordinate, is

$$\mu_1' = \frac{\Sigma(s_i S_i)}{N} \tag{16}$$

and the second moment, μ_2', is

$$\mu_2' = \frac{\Sigma(s_i^2 S_i)}{N} \tag{17}$$

The position of the true centroidal ordinate is

$$x_0 = s_0 - \mu_1' \tag{18}$$

and the true second moment, μ_2, is

$$\mu_2 = \mu_2' - (\mu_1')^2 \tag{19}$$

The second moment calculated in this manner has yet to be converted into absolute units by the relation

$$\mu_2^0 = \mu_2 \omega^2 \tag{20}$$

where ω is the distance between successive vertical chords, S_i, in centimeters. In the interest of greater accuracy it is advisable to chose ω, the class breadth, not too large, and as a rule, 20 to 30 divisions of s on either side of the center of the base line will be most satisfactory.

The diffusion constant calculated by any one of the methods given has to be corrected for photographic magnification and optical distances between camera, cell, and scale. If G is the photographic enlargement factor, l the optical distances from the lens to the scale, and b the optical distance from the center of the cell to the scale, the correction factor by which the calculated D values have to be multiplied is

$$F = \left(\frac{l - b}{lG}\right)^2 \tag{21}$$

The extent to which the experimental diffusion curves deviate from an ideal displacement distribution curve can be studied by transforming the coördinates s and S into normal coördinates, ξ and ψ, in which case the time of diffusion disappears as parameter.

The relations between s, S, ξ, and ψ are

$$\xi = (s - \mu_1')\frac{\omega}{\sigma} \tag{22}$$

and

$$\psi = S\frac{\sigma}{\omega N} \tag{23}$$

where σ is the calculated standard deviation, as discussed previously. The curve obtained in this manner is, for the ideal case, identical with the normal form of the ideal distribution curve, the values of which can be found in mathematical tables. The "fit" of these two curves is a measure of the homogeneity of the solute under investigation, and is illustrated for a monodisperse solution in figure 12 and for a polydisperse solution in figure 13.

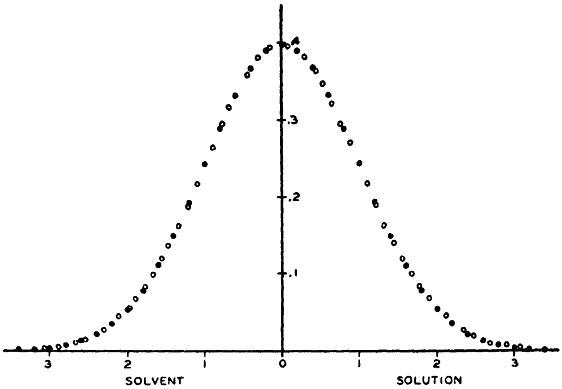

FIG. 12. Comparison of an ideal Gaussian distribution curve with the diffusion curve as obtained from measurements on a 0.8 per cent solution of urea-denatured serum albumin in 8 M urea (31). The open circles refer to the experimental points, plotted in normal coördinates. The full circles indicate the position of the ideal distribution curve.

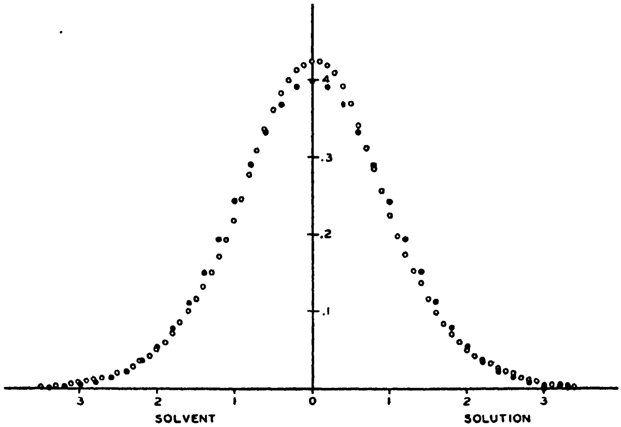

FIG. 13. Comparison of an ideal Gaussian distribution curve with the diffusion curve as obtained from measurements on a 0.8 per cent serum albumin solution at pH 7.6, denatured by heating for 30 min. at 70°C. Polydispersity is most clearly indicated by the difference in maximum ordinates of the ideal curve (full circles) and the experimental curve (open circles). Unpublished experiments by Neurath, Cooper, and Erickson.

While, in the preceding cases, the diffusion constant has been calculated from a single curve, it is, of course, also possible to obtain values of D from a comparison of the properties of the curves obtained after consecutive time intervals. It will be noted that in the equations given, the diffusion constant is related to any two of the variables H_i, X_i, and A, t being a constant. By introducing t as a variable, another set of equations can be formulated if any one of the above-mentioned variables is chosen as constant. This method has been used recently in studies of the diffusion of the rabbit papilloma virus protein (32), with the particular object of differentiating between lack of monodispersity, on one hand, and restriction of free diffusion, on the other, as the origin of the observed variations in apparent diffusion constant with protein concentration.

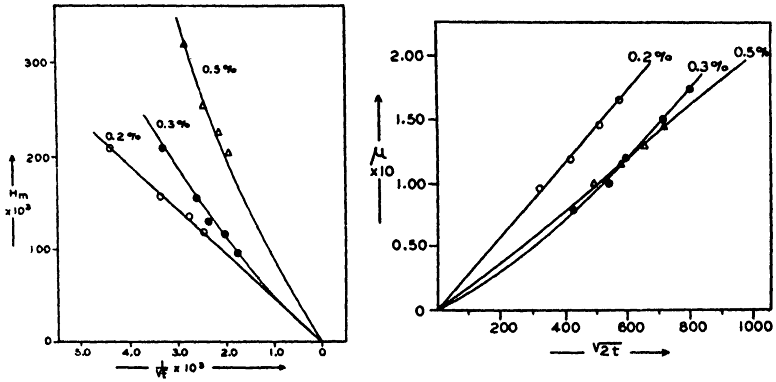

FIG. 14. Relation between time of diffusion and maximum ordinate, H_m (left), and half the distance between the inflection points, μ (right) of diffusion curves, as obtained from measurements on the rabbit papilloma virus protein (32). For further explanation see text and equations 24 and 25.

From equation 10, for instance, it follows that

$$\frac{\mu}{\sqrt{2t}} = \sqrt{D} \tag{24}$$

and

$$\frac{H_m}{\sqrt{t}} = \frac{K}{\sqrt{D}}, \quad \text{where } K = \frac{A}{2\sqrt{\pi}} \tag{25}$$

The results obtained from a plot of these equations are shown in figure 14. While in the most dilute solutions the curves are linear in both plots, diffusion anomalies occurring in protein concentrations of 0.3 and 0.5 per cent are evidenced both by the variations in the slope and by the non-linear shape of the curves.

(b) Polydisperse systems

While, with monodisperse solutions, calculations by any one of the methods just given yields a value for the true diffusion constant of the solute in question, average values are obtained if the solutions are polydisperse.

An estimate of the diffusion constant of the fastest moving component can be obtained with the aid of equation 11, provided its diffusion rate, relative to that of the other components, is sufficiently fast to establish a concentration gradient of its own, at higher values of x. In that case a set of equations of the form given in equation 11 can be set up, and $D_{\text{lim.}}$, the limiting value of the diffusion constant, extrapolated from $x \rightarrow \infty$, $H_i \rightarrow 0$. A more detailed discussion of the limitations of this method will be given in section D4 (page 378).

The problem of the resolution of a compound diffusion curve of a polydisperse system into its component curves has received attention recently by Dr. L. W. Nordheim and the author. Assuming a solution to be composed of two components of different diffusion constants, the individual constants and the concentrations of the two components may be calculated as follows:

The diffusion equation is as stated previously,

$$\frac{\mathrm{d}n}{\mathrm{d}x} = \frac{n_1 - n_0}{2\sqrt{\pi D t}} e^{-\frac{x^2}{4Dt}} \tag{26}$$

Substituting for $2Dt = \sigma^2$ and for $n_1 - n_0 = kc$,

$$\frac{\mathrm{d}n}{\mathrm{d}x} = \frac{c}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}} \tag{27}$$

For a solution[10] containing i components with concentrations c and standard deviations σ, the general diffusion equation is (16)

$$g(x) = \int_{\sigma=0}^{\infty} \frac{c(\sigma)}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}} \mathrm{d}\sigma \tag{28}$$

and for $i = 2$

$$g(x) = \frac{c_1}{\sigma_1\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma_1^2}} + \frac{c_2}{\sigma_2\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma_2^2}} \tag{29}$$

Calculation of higher moments and integration with respect to x yields the following set of equations:

$$\int_0^{\infty} g(x)\,\mathrm{d}x = \nu_0 = \frac{c_1}{\sigma_1\sqrt{2\pi}}\sigma_1\sqrt{\frac{\pi}{2}} + \frac{c_2}{\sigma_2\sqrt{2\pi}}\sigma_2\sqrt{\frac{\pi}{2}} = \frac{1}{2}(c_1 + c_2) \tag{30}$$

$$\int_0^{\infty} xg(x)\,\mathrm{d}x = \nu_1 = \frac{c_1}{\sigma_1\sqrt{2\pi}}\sigma_1^2 + \frac{c_2}{\sigma_2\sqrt{2\pi}}\sigma_2^2 = \frac{1}{\sqrt{2\pi}}(c_1\sigma_1 + c_2\sigma_2) \tag{31}$$

$$\int_0^{\infty} x^2 g(x)\,\mathrm{d}x = \nu_2 = \frac{c_1}{\sigma_1\sqrt{2\pi}}\sqrt{\frac{\pi}{2}}\sigma_1^3 + \frac{c_2}{\sigma_2\sqrt{2\pi}}\sqrt{\frac{\pi}{2}}\sigma_2^3 = \frac{1}{2}(c_1\sigma_1^2 + c_2\sigma_2^2) \tag{32}$$

$$\int_0^{\infty} x^3 g(x)\,\mathrm{d}x = \nu_3 = \frac{c_1}{\sigma_1\sqrt{2\pi}}2\sigma_1^4 + \frac{c_2}{\sigma_2\sqrt{2\pi}}2\sigma_2^4 = \frac{2}{\sqrt{2\pi}}(c_1\sigma_1^3 + c_2\sigma_2^3) \tag{33}$$

---

[10] Here it is assumed that dn/dc is constant not only for the whole solution but also for the individual components. If the specific refractive-index increments of the components are different, refractive indices have to be used in the calculations in place of concentrations. For proteins of closely related properties such as those derived from a mixture by fractional precipitation, the refractive-index increments may be considered to be the same.

Here ν_0 is equal to one-half the area under the curve, and ν_1, ν_2, and ν_3 to the first three moments calculated for either one of the halves of the curve, with the centroidal ordinate as origin.

Setting

$$a = \frac{1}{\nu_0} 2\nu_0 \tag{34}$$

$$b = \frac{\sqrt{2\pi}}{\nu_0} \nu_1 \tag{35}$$

$$c = \frac{1}{\nu_0} 2\nu_2 \tag{36}$$

$$d = \frac{\sqrt{2\pi}}{2\nu_0} \nu_3 \tag{37}$$

we obtain

$$\sigma_{1,2} = -\frac{1}{2}\frac{bc - ad}{ac - b^2} \pm \sqrt{\frac{1}{4}\left(\frac{bc - ad}{ac - b^2}\right) - \frac{bd - c^2}{ac - b^2}} \tag{38}$$

where σ_1 and σ_2 are the standard deviations of the two components, related to their respective diffusion constants by equation 14. Substitution of σ_1 and σ_2 into equations 31 and 32 yields the relative and absolute concentrations of the two components[11].

The method as presented here is strictly applicable only if the experimental diffusion curves are truly symmetrical and if the solutions under investigation are known to be composed of only two components. If there are more than two, this method yields only mean values which, however, are probably closer approximations than those obtained from a single standard deviation. The method should prove particularly useful when applied to protein solutions for which the presence of two components has been established by sedimentation analysis, since it facilitates the determination of the respective diffusion constants without previous separation and isolation of the individual components. A discussion of the limitations of the method will be given in section D4 (page 378).

The problem of the diffusion of polydisperse systems has also been considered recently by Gralén (9). His calculations are directed primarily toward an evaluation of the average diffusion constant. The type of average has been shown by him to depend on the method of calculation employed. Thus with equation 12 the average diffusion constant D_0 is

$$D_0^{A,H_m} = \left(\frac{\Sigma c_i}{\Sigma \frac{c_i}{\sqrt{D_i}}}\right)^2 \tag{39}$$

---

[11] Equation 33 may also be replaced by the relation

$$\frac{c_1}{\sigma_1} + \frac{c_2}{\sigma_2} = H_m$$

where H_m is the maximum ordinate. This method may be more convenient in that it eliminates the calculation of the third moments.

and with equation 14

$$D_0^{\sigma} = \frac{\Sigma c_i D_i}{\Sigma c_i} \tag{40}$$

where i is the number of diffusing components, c their respective concentration, and D the respective diffusion constant. The ratio of these two average diffusion constants

$$\frac{D_0^{\sigma}}{D_0^{A,H_m}} = \frac{\Sigma c_i D_i \left(\Sigma \frac{c_i}{\sqrt{D_i}}\right)^2}{(\Sigma c_i)^3} \tag{41}$$

is considered to be a measure of the degree of polydispersity. Assuming a logarithmic distribution function, Gralén calculates the distribution of diffusion constants in relation to the relative concentrations of the diffusing components.

4. *Resolving power and accuracy*

We have stated in the preceding discussion that, for a monodisperse solution, the diffusion constants calculated with the aid of the various equations should agree with one another to within 2 per cent or better. The question arises, however, whether such a close agreement is necessarily an indication of monodispersity or whether it may not merely simulate monodisperse behavior of an actually polydisperse system. This problem has, as far as we are aware, not been considered previously. Its importance for determinations of the state of dispersion of a protein solution and the determination of the molecular weights of proteins is obvious and can hardly be overemphasized.

The following discussion is limited to a comparison of a monodisperse solution, on one hand, with one composed of two diffusing entities, on the other. If in equation 29 one of the two right-hand members is small enough to be neglected in comparison with the other, the equation can be reduced to the logarithmic form given in equation 11. This is possible if (*1*) $\sigma_2 = 0$, in which case one is dealing with a monodisperse solution; (*2*) $\sigma_2 \ll \sigma_1$; or (*3*) $c_2 \ll c_1$. Case (*2*) implies that, as mentioned, at a given distance, x, the observed refractive-index gradient is due only to the first component and is zero for the second component. Case (*3*) implies merely that the concentration of the second component is altogether too small to give rise to a measurable refractive-index gradient. If, therefore, $\sigma_1 = \sigma_2$, or $\sigma_2 = 0$, values of D calculated with equation 11 should be constant over the whole region of the diffusion curves and the solution may be said to be monodisperse. If D is found to increase with increasing values of x, the limiting value of the diffusion constant of the fastest moving component can be determined by extrapolation to $x \rightarrow \infty$, provided condition (*2*) is known to be fulfilled. Otherwise, equation 11 is invalid and the method of "successive analysis" is not applicable.

The most readily detectable difference between the observed diffusion curves and normal Gaussian distribution curves exists with respect to their maximum ordinates. If, in equation 29, x is taken as zero, this equation reduces to

$$\left(\frac{dn}{dx}\right)_{x=0} = H_m^{1,2} = \frac{c_1}{\sigma_1\sqrt{2\pi}} + \frac{c_2}{\sigma_2\sqrt{2\pi}} \tag{42}$$

where $H_m^{1,2}$ is the maximum ordinate, and σ_1 and σ_2 are the standard deviations of the two components present in concentrations c_1 and c_2. σ, it will be recalled, is proportional to the square root of the diffusion constant.

For a monodisperse substance we have

$$H_m = \frac{c}{\sigma\sqrt{2\pi}} \tag{43}$$

Setting

$$c = c_1 + c_2$$

the maximum ordinate of a diffusion curve of a monodisperse solution can be compared with that of a curve produced by the simultaneous diffusion of two components. The quantity $\left(\frac{H_m^{1,2}}{H_m} - 1\right)100$ gives the percentage change in the maximum ordinate of the diffusion curve of a mondisperse solution. In table 1 values are given for this quantity, calculated for various ratios of D_2/D_1 in relation to assumed values of c_2/c_1. σ has been assumed to be equal to σ_1, i.e., we compare the maximum ordinate of the diffusion curve of a monodisperse solution of the faster diffusing component with that of a solution containing both components in varying relative concentrations.

It may be seen from table 1 that the maximum ordinate of the compound diffusion curve is always higher than that of the diffusion curve of component 1. The percentage increase is smaller the more closely the two diffusion constants approach each other and, for a given value of D_2/D_1, the lower the relative concentration of the second component. The accuracy with which the maximum ordinate can be determined experimentally is about 1 per cent (about ± 2 μ for a maximum scale-line displacement of 300 μ). Hence, it is practically impossible to detect with *this method* of calculation the presence of about 20 per cent of material having a diffusion constant 10 to 20 per cent lower than that of the major component; the resolving power increases, however, with increasing differences in the two diffusion constants, and with ratios of D_2/D_1 approaching a value of 0.5, the resolution becomes sufficiently distinct to detect the presence of the second component[12] in a concentration as low as about 5 per cent of that of the total protein.

---

[12] We have stated in a recent paper (30) that the diffusion measurements on serum albumin denatured by urea, or by guanidine hydrochloride, indicate the solutions to be monodisperse. The ratios of the diffusion constants of native and denatured protein are, in these cases, about 0.5, and the agreement between the observed and calculated maximum ordinates is within the limits of the experimental error (see figure 12). This conclusion is in accord with the present ideas.

The situation becomes somewhat different if the compound diffusion curve is compared with that of a single substance, the diffusion constant of which lies between those of the two components. Such a comparison is made in table 2 for the case in which the diffusion constant of the single substance, $\sqrt{D_0}$, is equal to $\frac{\sqrt{D_1} + \sqrt{D_2}}{2}$. The change in maximum ordinate is again expressed

TABLE 1

Comparison of the maximum ordinate of a compound diffusion curve of a mixture of two components of diffusion constants D_1 and D_2 with the maximum ordinate of the diffusion curve of a single component of diffusion constant D_1

c_1 and c_2 are the relative concentrations of components 1 and 2, D_2/D_1 is the ratio of their diffusion constants, and $\left(\frac{H_m^{1,2}}{H_m^1} - 1\right) \times 100$ is the percentage change in the maximum ordinate, H_m^1, of the diffusion curve of a monodisperse solution of component 1

c_1	c_2	PER CENT CHANGE IN H_m^1 FOR $\frac{D_2}{D_1}$ =					
		0.9	0.79	0.70	0.50	0.25	0.10
1	0	0	0	0	0	0	0
0.9	0.1	0.5	0.6	2	4.1	10	126
0.75	0.25	1.3	3.3	4.9	10.4	25	142
0.50	0.50	2.5	7.0	9.7	20.8	50	166
0.25	0.75	4.0	10.0	14.8	31.2	75	191
0.10	0.90	4.8	11.2	17.8	37.5	90	206
0	1.0	5.2	13.7	19.8	41.6	100	216

TABLE 2

Comparison of the maximum ordinate of a compound diffusion curve of a mixture of two components of diffusion constants D_1 and D_2 with the maximum ordinate of a diffusion curve for a single component of diffusion constant D_0, where $\sqrt{D_0} = \frac{\sqrt{D_1} + \sqrt{D_2}}{2}$

c_1 and c_2 are the relative concentrations of components 1 and 2; D_1 and D_2 are their diffusion constants; $\left(\frac{H_m^{1,2}}{H_m^0} - 1\right) \times 100$ is the percentage change in the maximum ordinate, H_m^0, of the diffusion curve of a monodisperse solution of a component with diffusion constant D_0 and concentration $c_0 = c_1 + c_2$

c_1	c_2	$\left(\frac{H_m^{1,2}}{H_m^0} - 1\right) \times 100$
		per cent
1	0	−14.7
0.90	0.10	−11.2
0.75	0.25	−5.9
0.50	0.50	+3.0
0.25	0.75	+11.9
0.10	0.90	+17.2
0	1.0	+20.8

in terms of percentage of that of the diffusion curve for the single substance, $\left(\frac{H_m^{1,2}}{H_m^0} - 1\right)100$. Here, it will be noted, the maximum ordinate of the compound curve is lower than that of the single curve for values of c_2 below 0.5 and higher for values above 0.5. The region near $c_2 = 0.5$ is of interest because the per cent deviation approaches zero, implying that it is impossible to decide, on the basis of the maximum ordinate alone, whether one is dealing with a mixture of two components or with a single component, the diffusion constant of which is about half-way between that of the two components.

Such an apparent uncertainty can be partly dispelled if, from the history of the experiment, predictions can be made regarding the values of the diffusion constants to be expected. However, the calculations given in tables 1 and 2 refer to particular cases, and it can be readily seen that equation 29 permits of an infinite number of solutions depending on the choice of the values for c_1, c_2, σ_1, and σ_2.

The problem becomes definite upon application of the statistical method of analysis. We have seen that the four equations (30 to 33) contain four unknowns and, therefore, are capable of one solution only. As a test for monodispersity, the following relations, which follow directly from these equations, may be applied:

$$\nu_0 \nu_1 \geqq (\nu_1)^2 \frac{\pi}{2} \tag{44}$$

and

$$\nu_0 \nu_3 \geqq 2 \nu_1 \nu_2 \tag{45}$$

The right-hand members of these equations should be equal to the left-hand members for a monodisperse solution, and smaller for a two-component solution. Moreover, the absolute values of the moments will be different for a monodisperse solution, on one hand, and for a polydisperse solution, on the other. An illustration of these relations is given in table 3. The symbols have the same meaning as in the corresponding equations. Column A refers to a 1:1 mixture of materials the diffusion constants of which are assumed to be 7×10^{-7} and 5×10^{-7}, column B to a 1:1 mixture, diffusion constants 7×10^{-7} and 3.5×10^{-7}, and column C to a single substance, diffusion constant 7×10^{-7}. In the first case, the per cent difference is 0.8 for equation 44, and 1.5 for equation 45; in the second case, the corresponding differences are 3.5 per cent and 5.6 per cent. While the former values are probably too small to be detected experimentally, the latter are outside the limits of the experimental error. The differences between the absolute values calculated for the three cases are very marked and may be used as a guide when other evidence is available to indicate an expected value for D or c.

In summarizing this discussion, it may be stated that application of diffusion measurements to the determination of the state of dispersion of a protein solution is limited by two factors, one of theoretical, the other of practical nature. The former has to do with the insensitivity of Gaussian distribution curves to small variations in the standard deviation; the latter is related to the accuracy with which the curves can be obtained experimentally, even when the greatest care is exercised. There is, however, little doubt that the application of the above relations to experimental data should place diffusion measurements on a much more reliable basis than they appear to be at present.

TABLE 3

Comparison of statistical properties of diffusion curves of monodisperse and polydisperse solutions

D_1, D_2 = diffusion constants of components 1 and 2; c_1, c_2 = relative concentrations of both components; t = time of diffusion in seconds; ν_0, ν_1, ν_2, ν_3 = moments of diffusion curves (for their meaning see text); Δ_{I} = difference between values listed in rows 10 and 11, expressed in per cent of value listed in row 10; Δ_{II} = difference between values listed in rows 13 and 14, expressed in per cent of value listed in row 13

	PROPERTIES	A	B	C
(1)	$D_1 \times 10^7$	7	7	7
(2)	$D_2 \times 10^7$	5	3.5	
(3)	t in seconds	86,400	86,400	86,400
(4)	c_1	0.5	0.5	1.0
(5)	$c_2 = 1 - c_1$	0.5	0.5	0
(6)	ν_0	0.5	0.5	0.5
(7)	ν_1	0.1280	0.1184	0.1387
(8)	ν_2	0.0519	0.0454	0.0605
(9)	ν_3	0.0270	0.0228	0.0337
(10)	$\nu_0\nu_2$	0.0260	0.0227	0.0302
(11)	$(\nu_1)^2\frac{\pi}{2}$	0.0258	0.0220	0.0302
(12)	Δ_{I}	0.8	3.5	0
(13)	$\nu_0\nu_3$	0.0135	0.0114	0.0168
(14)	$2\nu_1\nu_2$	0.0133	0.0107	0.0168
(15)	Δ_{II}	1.5	5.6	0

III. RESULTS

The diffusion constants of a large number of proteins have been measured in recent years. The impetus to these determinations came largely from sedimentation-velocity measurements, which require a knowledge of the diffusion constants for the determination of molecular weights. Measurements with the light-absorption and the light-refraction methods have been carried out most extensively in Svedberg's laboratory, where these methods were originated, in the laboratory of J. W. Williams, and in that of the present author. Before considering these results it may be of interest to compare the values of diffusion constants as obtained for certain proteins by the various experimental methods previously discussed. This is done in table 4.

The results obtained with the porous-disk method are more susceptible to experimental error than those obtained from measurements of the absolute diffusion rate, for the reasons already given. The discrepancies to be noted in table 4 are probably due to a large extent, to differences in the preparation of the materials employed by the various investigators. It would be of interest to see how well the results would agree if one and the same material were to be used for comparative measurements.

Sufficient material has accumulated to make it possible to estimate the precision of the refractometric-scale method. Lamm's (16) measurements on potassium chloride and pentaerythritol, analyzed by means of the methods of calculation considered previously (section D3), reveal a standard deviation from the mean not exceeding 1 per cent. With monodisperse protein preparations such as egg albumin (42), excelsin (42), serum albumin (29), serum pseudoglobulin (29), and others, the standard deviation from the mean lies close to 2 to 3 per cent.

TABLE 4

Comparison of the diffusion constants of certain proteins as determined by various methods of measurement

$D^{25°}_{H_2O}$ = diffusion constant calculated for 25°C. in water

PROTEIN	T	METHOD	$D^{25°}_{H_2O}$	REFERENCE
	°C.		*cm.² per second × 10⁷*	
Pepsin	8	Porous disk	8.9*	(35)
	20	Scale	10.3†	(42)
	25	Scale	10.0, 9.2‡	(29)
Hemoglobin (carbon monoxide)	5	Porous disk	6.9*	(36)
	0	Porous disk	8.0	(10)
	20	Light absorption	7.2	(53)
	20	Scale	7.9	(42)
Egg albumin	25	Porous disk	11.1§	(21)
	20	Light absorption	8.7	(53)
	20	Scale	8.7	(42)
	0	Schlieren scanning	8.7¶	(18)

* Corrected for erroneous value of the diffusion constant of 0.1 N hydrochloric acid originally used in the calibration of the cell (2).

† Unfractionated crystalline preparation used by the investigator.

‡ The two values refer to two crystalline fractions differing from one another in solubility.

§ This value may be in error, since the measurements were carried out in the absence of salts.

¶ Extrapolated to infinite dilution according to Polson's data (42).

In the following, we shall examine the factors which have been found to influence the diffusion rate of proteins and which, unfortunately, have not always received the attention they deserve. The most important of these factors are the time of diffusion, the temperature, the solvent viscosity, and the solute concentration.

A. TIME OF DIFFUSION

While the majority of measurements that have been published do not reveal any significant drift of the apparent diffusion constant with time, there has been noted in several instances a gradual decrease of the diffusion rate, approaching asymptotically a constant value after longer time intervals.

This effect has been observed with both the porous-disk method (10, 57) and the refractometric-scale method. Of the monodisperse proteins studied by Polson (42), about one-fourth revealed a downward drift of D with time, whereas the remainder did not. Of the experiments carried out in the author's laboratory, about one-half exhibited this effect. With secalin, studied by Andrews (1), D decreased to as much as one-fourth of the initial value. Longsworth's recent measurements on egg albumin[13] show a downward drift at pH 4.64 but not at pH 11.81. The origin of this effect is rather obscure and its elucidation must await further detailed measurements on monodisperse solutions; it also remains to be seen whether in experiments where this effect is observed the mean value or the limiting value should be taken as the best approximation to the true diffusion constant.

B. TEMPERATURE

The influence of temperature on the diffusion constant is given by the equation

$$\frac{D_x}{D_y} = \frac{T_x}{T_y}\frac{\eta_y}{\eta_x} \tag{46}$$

where D_x and D_y are the diffusion constants at the absolute temperatures T_x and T_y, respectively, and η_x and η_y the corresponding viscosities of the solvent. The equation has been verified experimentally by Tiselius and Gross for phycoerythrin (53) (measured at 20° and 30°C.) and by Polson for egg albumin (42) (measured at 15°, 20°, and 25.1°C.). Longsworth's measurements on egg albumin (18), carried out at 0°C., likewise agree with Polson's data when corrected for the temperature by means of equation 46.

C. VISCOSITY

While this factor will be considered more fully in the following paragraph, it may be said here that, by and large, the viscosity correction

$$D_x = D_y\frac{\eta_y}{\eta_x} \tag{47}$$

holds, for values of η_y/η_x not exceeding 1.1. Here D_x and D_y are the diffusion constants measured at a constant temperature in solvents of viscosities η_x and η_y, respectively. Diffusion constants are usually referred to water as a solvent, in which case η_x is the water viscosity at a given temperature (20° or 25°C.) and η_y that of the solvent into which diffusion takes place.

Polson's measurements on egg albumin (42), in ammonium sulfate solutions, varying in relative viscosity (with respect to water) between 1.090 and 1.437, indicate, however, a slight increase in the apparent diffusion constant when corrected for the solvent viscosity by means of equation 47; recent measurements on serum albumin in solutions of sucrose, as well as in solutions of urea and guanidine hydrochloride (30) (η/η_0 varying between 1.15 and 1.30), cast some doubt on the strict validity of equation 47. In these instances, the relative viscosity influential in retarding the diffusion rate appears to be about 10 per cent higher than that actually measured. This problem is being further investigated in the author's laboratory.

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[13] The author is indebted to Dr. L. G. Longsworth for placing these data at his disposal.

D. THE INFLUENCE OF CONCENTRATION ON THE DIFFUSION RATE

We have mentioned at the outset that the validity of the diffusion equation 3 requires the independent motion of the diffusing entities; under these conditions the diffusion rate is independent of the solute concentration. While on a molar basis, protein solutions of 1 to 5 per cent concentration are still in the "infinite dilution" range, yet the diffusion rate is not independent of concentration. This non-ideal behavior is reflected also by sedimentation and viscosity and is frequently met with in solutions of high-molecular-weight compounds.

The influence of concentration on the diffusion rate may be studied by either one of the following methods: (*1*) the initial concentration difference is varied by increasing the concentration of the solution and keeping that of the solvent at zero; (*2*) the initial concentration difference is kept constant, but the absolute concentration of both solution and "solvent" is increased. The first method is limited in scope by the optical disturbances occurring with high refractive-index gradients, as discussed in section D2 (page 22). It can be used up to protein concentrations of about 1.5 per cent.

Where a concentration dependency of the diffusion constant, within the limits of about 0.2 and 1.5 per cent, is observed, the true diffusion constant can be determined by extrapolation to zero concentration. This is analogous to the procedure used in other physical measurements, such as those of osmotic pressure or sedimentation rate. Of course, this method is restricted in applicability if, for instance, particle dissociation occurs in very dilute solutions, as has been observed with hemoglobin (40). A protein the diffusion rate of which is fairly independent of the concentration up to about 1.4 per cent is egg albumin, as can be seen from table 5. With the tobacco mosaic virus protein (33), on the other hand, the diffusion constant is concentration dependent in concentrations as low as 0.2 per cent. This is evidenced by the variations in the calculated values of the diffusion constant, and also by the shape of the individual diffusion curves. They are skewed, as shown in figure 15, and the position of the maximum ordinate shifts with time toward regions of lower concentration. These phenomena have been related to a restriction of the diffusion rate in the regions of relatively high concentration, owing to interparticle attraction, and to a corresponding acceleration in regions of relatively lower protein concentration (33).

In the second method for studying the influence of concentration on the diffusion rate, the absolute solute concentrations are varied in both solute and "solvent," while their relative concentrations remain constant. Thus, the diffusion of a 1 against a 0 per cent solution, of a 2 against a 1 per cent solution, or of a 6 against a 5 per cent solution should proceed with equal rate if the diffusion constant is concentration independent. Such measurements have been carried out by Polson (42) on six different proteins, varying in molecular weight between 17,000 and 750,000, and in concentrations ranging from 1 to 9 per cent.

TABLE 5

Diffusion constant of egg albumin in relation to protein concentration (Polson (42))

PROTEIN CONCENTRATION	$D_{H_2O}^{20°}$
per cent	$\times 10^7$
1.4	7.64
0.91	7.71
0.88	7.76
0.83	7.73
0.7	7.71

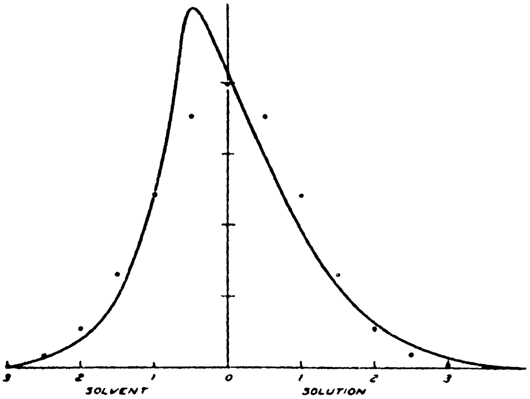

FIG. 15. Comparison of an ideal Gaussian distribution curve with the diffusion curve as obtained from measurements on a 1 per cent solution of tobacco mosaic virus protein. The open circles indicate the position of the ideal curve; the solid line refers to the experimental curve plotted in normal coördinates (33).

In evaluating his results, Polson assumes that the frictional resistance effective in retarding the diffusion rate is only that of the buffer solution in which the proteins were dispersed, whereas the protein molecules contained in the "solvent" do not contribute to the friction. Thus, in systems in which, for instance, a buffered solution containing 6 per cent of protein diffuses against a buffered 5 per cent solution of the same protein, the viscosity correction is assumed to be $\eta_{\text{buffer}}/\eta_{H_2O}$, i.e., the same as that employed in cases where the protein diffuses into the buffer itself. On this basis, Polson arrived at the conclusion that with some proteins, such as hemocyanin *Homarus*, the diffusion constant increases very slightly with increasing protein concentration, and that with amandin it remains constant, whereas with serum globulin, egg albumin, lactoglobulin, and erythrocruorin *Lampetra* it decreases as the concentration is increased. Polson's data are tabulated in column 3 of table 6 and are compared with the respective values of D_∞, the limiting value of the diffusion constant obtained by extrapolation to zero concentration[14] (column 7 of table 6).

If, however, one applies the full correction for the viscosity of the solution into which diffusion takes place, $\left(\frac{\eta_{x \text{ per cent protein}}}{\eta_{H_2O}}\right)$, values are obtained as given

TABLE 6

Diffusion in concentrated solutions

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
SOLUTE	CONCENTRATION		$D^{20} \times 10^7$	η/η_0 SOLVENT	$D^{20} \times \frac{\eta}{\eta_0} \times 10^7$	D^{20}_∞ (OBSD.) $\times 10^7$	$\frac{D^{20}}{D^{20}_\infty}$	D^{20}_∞ (CALCD.)
	Solution	Solvent						
Egg albumin (42)*	3	2	7.43	1.089	8.14	7.76	0.958	7.74
	6	5	6.94	1.240	8.62	7.76	0.884	7.77
	9	8	6.11	1.48†	9.05	7.76	0.787	7.78
Lactoglobulin (42)	5.5	5	6.46	1.246	8.05	7.30	0.885	7.26
Serum albumin (5)	5	4	5.54	1.213	6.72	6.11	0.907	6.11
	6.7	5.7	5.20	1.348†	7.00	6.11	0.850	6.15
Sucrose (42)	5	4.5	42.8	1.116‡	47.8	45.6	0.939	45.1
	10	9	39.9	1.280‡	51.0	45.6	0.875	45.6
Sucrose in 4 per cent serum albumin (5)	1	0	41.4	1.213	52.2	45.6	0.907	45.7
Amandin (42)	4.5	4	3.55	1.23	4.37	3.62	0.98	3.95
	8.66	8	3.57	1.64	5.83	3.62	0.98	5.00
Hemocyanin *Homarus* (42)	3.4	2.5	2.80	1.13	3.16	2.78	1.00	2.98
	6.8	5.9	2.83	1.36	3.84	2.78	1.02	3.36
Hemoglobin *Lampetra* (42)	1.66	1.08	10.17	1.05§	10.68	10.65	0.955	10.4
	3.33	2.71	9.34	1.13§	10.58	10.65	1.02	9.95

* The numbers given in parentheses refer to the observer as listed in the bibliography.
† Extrapolated values. ‡ Interpolated value. § Calculated from the dissymmetry constant with the aid of the Perrin and Simha equations (29).

in column 6 of table 6. It will be noted that, according to these calculations, the apparent diffusion constant increases with increasing protein concentration. Such could only be the case if the molecules either dissociate into smaller units, or become more nearly spherical in shape. The fact, however, that a simple substance like sucrose follows the same trend, together with the known stability of some of these proteins, discredits such a hypothesis and calls for a closer examination of the problem. In its broadest form, it can be stated as follows: What is the frictional resistance encountered by a molecule when it diffuses through a medium containing molecules of its own kind or molecules of comparable size? We have seen in the preceding discussion that the solvent can, by and large, be considered as continuum as long as the solvent molecules are small in comparison with those of the solute; in that case, the simple viscosity correction as given in equation 47 is applicable. The data given in table 6 demonstrate that, in the present case, the calculated values for the diffusion constant are too high if the full viscosity correction is applied, and too low, if the contribution of the large molecules to the frictional resistance is neglected.

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[14] While Polson believes that the concentration dependency of a protein is directly related to its molecular volume (the larger the volume, the smaller the interparticle distance and the greater the concentration dependency), it appears to us that such a viewpoint neglects many other factors which are known to influence the interaction between molecules of colloidal dimensions. Thus, for instance, the shape of the molecules and the nature of their surface undoubtedly play an important rôle. This can be seen from a comparison of the diffusion behavior of the tobacco mosaic virus protein (33), on one hand, and of the rabbit papilloma virus protein on the other (32). Both have molecular weights of comparable magnitude, but the concentration dependency of the diffusion constant of the rod-shaped tobacco mosaic virus protein molecules is considerably greater than that of the more nearly symmetrically shaped molecules of the rabbit papilloma virus protein.

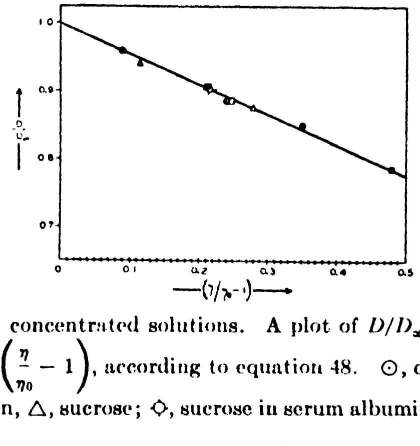

FIG. 16. Diffusion in concentrated solutions. A plot of D/D_∞ against the specific viscosity of the "solvent" $\left(\frac{\eta}{\eta_0} - 1\right)$, according to equation 48. ⊙, egg albumin; □, lactoglobulin; ●, serum albumin, △, sucrose; ◇, sucrose in serum albumin. Compare also table 6.

A thorough discussion of the problem would greatly exceed the scope of this review; however, it may be of interest to present here an empirical relation which, in the course of a preliminary investigation, Dr. G. R. Cooper and the author have found to be applicable to the diffusion of protein into protein, of sucrose into sucrose, and of sucrose into protein (5). In all these cases, the diffusing molecules are either of *comparable* size or *smaller* than those contained in the medium into which diffusion occurs. The experimental data employed were those of Polson (42), as well as unpublished measurements carried out in this laboratory on the diffusion of concentrated serum albumin solutions, and on the diffusion of 1 per cent sucrose dispersed in a 4 per cent serum albumin solution and diffusing into the 4 per cent albumin solution.

A linear relation was obtained when D/D_∞ was plotted against $\left(\frac{\eta}{\eta_0} - 1\right)$, where D is the measured diffusion constant, D_∞ its limiting value for infinite dilution, and $\left(\frac{\eta}{\eta_0} - 1\right)$ the specific viscosity of the medium into which diffusion took place. The data are plotted in figure 16. In this plot, only those monodisperse proteins have been considered for which the observed diffusion constant decreases with increasing concentration. Polson's data for serum globulin were omitted on account of the polydisperse nature of the preparation employed by him. The straight line drawn through the points in figure 16 follows the equation

$$\frac{D}{D_\infty} = 1 - k\left(\frac{\eta}{\eta_0} - 1\right) \tag{48}$$

where k turned out to be 0.446. In column 9 of table 6, there are given values of D/D_∞, calculated by means of equation 48. Comparison with the D_∞ values given in column 7 of this table indicates a very satisfactory agreement between these two sets of data. The physical meaning of equation 48 is as yet obscure. It will be recalled that $\left(\frac{\eta}{\eta_0} - 1\right)$, the specific viscosity of a solution, is proportional to its volume concentration; hence, the equation may also be written as

$$\frac{D}{D_\infty} = 1 - k'\phi \tag{49}$$

where ϕ is the ratio of the volume of the dispersed phase to that of the dispersion medium[15].

While no theoretical explanation will be offered at this time, nevertheless it is remarkable that this equation is equally valid for the high-molecular-weight proteins as well as for the low-molecular-weight sucrose. This fact suggests the equation to be of universal application. When Polson's measurements on hemocyanin *Homarus* and amandin are evaluated with our equation, one finds an increase of the apparent diffusion constant with increasing protein concentration. This might be due either to a splitting of the molecules, or to a decrease in molecular asymmetry without concomitant changes in size. The former interpretation appears to be more likely; Pedersen has found protein–protein interaction to cause, with certain proteins, dissociation into smaller units (40), and the hemocyanins in general, and amandin in particular, are known to be susceptible to dissociation. Moreover, the dissymmetry constants of both proteins in the native state are relatively low and could not decrease to the extent required by the increase in diffusion constant if the effects were ascribed entirely to changes in molecular shape.

With *Lampetra* hemoglobin, the observed decrease in diffusion constant is larger than that predicted by equation 48, suggesting, on the basis of the present considerations, an increase either in molecular asymmetry, or in molecular size, or both.

The empirical equation 48 may conceivably find application also to sedimentation-velocity measurements. Here, too, the sedimenting molecules are moving through a medium containing molecules of their own kind and should thus be affected by their frictional resistance. While in very dilute solutions this effect can be neglected, it should become significant in more concentrated solutions.

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[15] For solutions of spherical particles, k should be $1/2.5 = 0.4$. The close agreement between that value, and the value of 0.446 obtained here, is probably fortuitous.

The absolute values for the diffusion constants of proteins vary between about 1×10^{-6} and 1×10^{-7}. With the large virus proteins, considerably lower values have been found, about 3×10^{-8} for tobacco mosaic virus protein and 6.4×10^{-8} for the rabbit papilloma virus protein, for instance. In table 7 are summarized

TABLE 7

*Observed diffusion constants and calculated molecular weights and dissymmetry constants of certain proteins**

PROTEIN	$D^{25°}_{H_2O} \times 10^7$	M	f/f_0	REFERENCE
Ribonuclease	13.6†	12,700	1.04	(43)
Pepsin B	10.0			(29)
A	9.2			(29)
Oxytocic pressure hormone	8.5	30,000	1.18	(54)
Horse globin	7.5	37,000	1.47	(8)
Horse serum albumin (McMeekin)	7.4	69,000‡	1.21	(29)
Horse hemoglobin (recombined)	7.2	69,000	1.23	(8)
Diphtheria toxin	7.1	70,000	1.22	(41)
Horse serum albumin (Hewitt)	7 0	73,000‡	1.25	(29)
Digested antitoxin (diphtheria)	6 7	98,000	1.14	(41)
Myogen	5.5	150,000	1.26	(7)
Catalase	5.2			(47)
Secalin	4.8			(1)
Horse pseudoglobulin GI	4.6	174,000‡	1.42	(29)
GII	4.8	170,000‡	1.39	(29)
Antitoxin (diphtheria)	4.4	150,000	1.4	(38)
Horse serum albumin denatured				
by 8 *M* urea	4.1	78,000‡	1.86	(30)
by 8 *M* guanidine hydrochloride	3.7	81,000‡	2.03	(30)
Horse pseudoglobulin GII denatured				
by 5.6 *M* guanidine hydrochloride	4.0	75,000‡	1.95	(31)
by 8 *M* urea	3.0	170,000‡	1.95	(31)
Myogen denatured by 6 *M* urea	2.7	72,000	3.2	(7)
Tomato bushy stunt virus	1.2	10,000,000	1.27	(28)
Calf thymus nucleohistone	1.2	2,300,000	2.5	(4)
Rabbit papilloma virus	0.59	47,000,000	1.49	(32)
Tobacco mosaic virus	0.3§	60,000,000§	2.5§	(33)

* Including only those data that have been reported since the publication of Svedberg and Pedersen's monograph (49). For previous work see Table 48 of the monograph.

† Determined with the Tiselius electrophoresis apparatus.

‡ Calculated from diffusion and viscosity data.

§ This value is tentative, in view of the nature of the preparation employed in diffusion measurements, and in view of the observed diffusion anomalies.

the results of the most important diffusion measurements that have been reported since the publication of Svedberg and Pedersen's monograph (49). Unless otherwise indicated, the refractometric-scale method was used for these measurements. There are given also in this table values for the molecular weights and dissymmetry constants of the proteins in question.

IV. APPLICATIONS

The information that can be obtained from diffusion measurements on proteins is threefold. In the first place, the size of the protein molecules may be obtained directly from diffusion data if the molecules are known to be spherical in shape; if they are non-spherical, diffusion measurements have to be supplemented by some other physical or chemical method which furnishes a measure of a second parameter of the dimensions of the molecules. In the second place, it is possible to determine from changes in the diffusion rate any changes in the size or shape of the molecules which may have occurred as a result of chemical or physical reactions. Lastly, diffusion measurements yield an estimate of the degree of homogeneity of a protein solution.

The relation between diffusion constant and molecular dimensions has been reviewed on various recent occasions (20, 25, 26, 29, 37) and need not be considered here in any detail. The relation between diffusion constant and frictional coefficient is, generally,

$$D = \frac{RT}{f} \tag{50}$$

and for spherical molecules,

$$D_0 = \frac{RT}{f_0} \tag{51}$$

where

$$f_0 = 6\pi\eta N r$$

N is the Avogadro number, η is the viscosity of the solvent, and r is the molecular radius. For spherical molecules, the diffusion constant is, therefore, related to the molecular weight by

$$D = \frac{RT}{6\pi\eta N}\left(\frac{3MV}{4\pi N}\right)^{-1/3} \tag{52}$$

If the molecules are non-spherical, we have the relation

$$f = \frac{M(1 - V\rho)}{s} = \frac{RT}{D} \tag{53}$$

and

$$M = \frac{RTs}{D(1 - V\rho)} \tag{54}$$

This equation is usually employed for molecular-weight determinations from combined diffusion and sedimentation-rate measurements. Here s is the sedimentation constant, V the partial specific volume of the solute, and ρ the density of the solvent. Molecular weights can also be determined from diffusion measurements combined with any method which yields a value for f/f_0, the dissymmetry constant. The corresponding relation is

$$M = K\frac{1}{D^3 V}\left(\frac{f_0}{f}\right)^{1/3} \tag{55}$$

where

$$K = \frac{R^3 T^3}{162\pi^2 \eta^3 N^2}$$

The dissymmetry constant is related to the apparent molecular shape, as expressed by the ratio of a major to a minor axis of a prolate or oblate ellipsoid of revolution, by the Perrin equation; hence, any method that relates to the apparent molecular shape can be used, in conjunction with diffusion measurements, for molecular-weight determinations. It has already been pointed out (29) that values obtained in this manner are essentially independent of the degree of hydration.

Combined diffusion and viscosity measurements have been carried out in this laboratory on a large number of native and denatured proteins, and the agreement between the values obtained in this manner and those obtained by other methods, such as those of osmotic pressure or combined diffusion and sedimentation, is rather satisfactory (29, 30, 31). Measurements of the dielectric dispersion or double refraction of flow (37) can also be used for an estimation of the molecular shape and, in combination with diffusion data, of the molecular weight.

If the molecular weight of a protein is known, diffusion measurements lend themselves to an estimation of the dissymmetry constant, and hence of the *apparent* molecular shape. This term has been used by us to denote the shape that is calculated if the influence of hydration is neglected (25). It should be emphasized here that it was never inferred that the influence of hydration is of a negligibly small order of magnitude; rather, these calculations were made merely to show what kind of result is obtained if the dissymmetry factor is interpreted solely in terms of molecular asymmetry. The estimate of about 33 per cent hydration made in more recent calculations (29) appears to represent a good approximation. The relation between hydration and molecular asymmetry has been considered by Kraemer (12, 13) and others (29, 37) and need not be discussed here. It is only in the case of spherical shape that the degree of hydration can be estimated from diffusion data, in which case (12, 13)

$$\frac{D_0}{D} = \left(\frac{V_a + rV_1}{V_1}\right)^{1/3} \tag{56}$$

where D is the observed diffusion constant, D_0 that calculated for a spherical anhydrous molecule, V_a the partial specific volume of the anhydrous protein, V_1 that of the solvent, and r the amount of solvent combined with 1 g. of anhydrous protein. This equation assumes the density of the adsorbed solvent to be the same as that of the liquid in bulk.

This approach to the general problem of the relation between diffusion constant and molecular dimensions contains a number of theoretical approximations and uncertainties which call for further attention. The brief discussion has been given merely to illustrate the importance of diffusion measurements for an estimation of the dimensions of protein molecules.

Many of the ideas expressed in this paper have evolved as a result of discussions with Dr. L. W. Nordheim of the Department of Physics, Duke University, and with Dr. G. R. Cooper and Mr. John O. Erickson of this Department. The author is also indebted to the Rockefeller Foundation and to the Lederle Laboratories, Inc., for financial support which has made the work carried out in this laboratory possible.

REFERENCES

(1) ANDREWS, A. C.: J. Am. Chem. Soc. **62**, 942 (1940).
(2) ANSON, M. L., AND NORTHROP, J.: J. Gen. Physiol. **20**, 575 (1936-37).
(3) BOURDILLON, J.: J. Gen. Physiol. **24**, 459 (1941); **25**, 263 (1941).
(4) CARTER, R. O.: J. Am. Chem. Soc. **63**, 1960 (1941).
(5) COOPER, G. R., AND NEURATH, H.: Unpublished data.
(6) GLASSTONE, S., LAIDLER, K. J., AND EYRING, H.: *The Theory of Rate Processes.* McGraw-Hill Book Company, Inc., New York (1941).
(7) GRALÉN, N.: Biochem. J. **33**, 1342 (1939).
(8) GRALÉN, N.: Biochem. J. **33**, 1907 (1939).
(9) GRALÉN, N.: Kolloid-Z. **95**, 188 (1941).
(10) HAND, D. B.: J. Am. Chem. Soc. **61**, 3180 (1939).
(11) KINCAID, J. F., EYRING, H., AND STEARN, A. E.: Chem. Rev. **28**, 301 (1941).
(12) KRAEMER, E. O.: J. Franklin Inst. **229**, 393, 680 (1940).
(13) KRAEMER, E. O.: In T. Svedberg and K. O. Pedersen's *The Ultracentrifuge*, p. 57, ff. Oxford University Press, London (1940).
(14) KUNITZ, M., AND NORTHROP, J.: J. Gen. Physiol. **18**, 433 (1934-35).
(15) LAMM, O.: Z. physik. Chem. **A138**, 313 (1928); **A143**, 177 (1929).
(16) LAMM, O.: Nova Acta Regiae Soc. Sci. Upsaliensis [4] **10**, No. 6 (1937).
(17) LAMM, O., AND POLSON, A.: Biochem. J. **30**, 538 (1936).
(18) LONGSWORTH, L. G.: Ann. N. Y. Acad. Sci. **41**, 267 (1941).
(19) LOUGHBOROUGH, D. L., AND STAMM, A. J.: J. Phys. Chem. **40**, 1113 (1936).
(20) LUNDGREN, H. P., AND WILLIAMS, J. W.: J. Phys. Chem. **43**, 988 (1939).
(21) MCBAIN, J. W., DAWSON, C. R., AND BARKER, H. A.: J. Am. Chem. Soc. **56**, 1021 (1934).
(22) MCBAIN, J. W., AND LIU, T. H.: J. Am. Chem. Soc. **53**, 59 (1931).
(23) MEHL, J. W., AND SCHMIDT, C. L. A.: University of California Publications in Physiology **8**, 165 (1937).
(24) NEURATH, H.: Cold Spring Harbor Symposia Quant. Biol. **6**, 196 (1938).
(25) NEURATH, H.: J. Am. Chem. Soc. **61**, 1841 (1939).
(26) NEURATH, H.: Cold Spring Harbor Symposia Quant. Biol. **8**, 80 (1940).
(27) NEURATH, H.: Science **93**, 431 (1941).
(28) NEURATH, H., AND COOPER, G. R.: J. Biol. Chem. **135**, 455 (1940).
(29) NEURATH, H., COOPER, G. R., AND ERICKSON, J. O.: J. Biol. Chem. **138**, 411 (1941).
(30) NEURATH, H., COOPER, G. R., AND ERICKSON, J. O.: J. Biol. Chem. **142**, 249 (1942).
(31) NEURATH, H., COOPER, G. R., AND ERICKSON, J. O.: J. Biol. Chem. **142**, 265 (1942).
(32) NEURATH, H., COOPER, G. R., SHARP, D. G., TAYLOR, A. R., BEARD, D., AND BEARD, J. W.: J. Biol. Chem. **140**, 293 (1941).

(33) Neurath, H., and Saum, A. M.: J. Biol. Chem. **126**, 435 (1938).

(34) Neurath, H., and Saum, A. M.: J. Biol. Chem. **128**, 347 (1939).

(35) Northrop, J.: J. Gen. Physiol. **13**, 739 (1929–30).

(36) Northrop, J., and Anson, M. L.: J. Gen. Physiol. **12**, 543 (1928–29).

(37) Oncley, J. L.: Ann. N. Y. Acad. Sci. **49**, 121 (1941).

(38) Pappenheimer, A. M., Jr., Lundgren, H. P., and Williams, J. W.: J. Exptl. Med. **71**, 247 (1940).

(39) Pearson, K.: Phil. Trans. London **185A**, 71 (1894).

(40) Pedersen, K. O.: In T. Svedberg and K. O. Pedersen's *The Ultracentrifuge*, p. 356. Oxford University Press, London (1940).

(41) Petermann, M. L., and Pappenheimer, A. M., Jr.: J. Phys. Chem. **45**, 1 (1941).

(42) Polson, A.: Kolloid-Z. **87**, 149 (1939); **88**, 51 (1939).

(43) Rothen, A.: J. Gen. Physiol. **24**, 203 (1940).

(44) Saum, A. M.: Thesis, Cornell University, 1938.

(45) Scherp, H. W.: J. Gen. Physiol. **16**, 795 (1932–33).

(46) Stern, K. G.: Z. physiol. Chem. **217**, 237 (1933).

(47) Sumner, J. B., Dounce, A. L., and Frampton, V. L.: J. Biol. Chem. **136**, 343 (1940).

(48) Svedberg, T.: Kolloid-Z. (Ergänzungsband) **36**, 53 (1925).

(49) Svedberg, T., and Pedersen, K. O.: *The Ultracentrifuge.* Oxford University Press, London (1940).

(50) Svensson, H.: Kolloid-Z. **81**, 181 (1939); **90**, 141 (1940).

(51) Thoevert, J.: Ann. chim. phys. [7] **26**, 366 (1902).

(52) Thoevert, J.: Ann. phys. [9] **2**, 369 (1914).

(53) Tiselius, A., and Gross, D.: Kolloid-Z. **66**, 12 (1934).

(54) Van Dyke, H. B., Chow, B. F., Greep, R. O., and Rothen, A.: Am. J. Physiol. **133**, 473 (1941).

(55) Wiener, O.: Ann. Physik Chem. [N.F.] **49**, 105 (1893).

(56) Williams, J. W., and Cady, L. C.: Chem. Rev. **14**, 171 (1934).

(57) Zeile, K.: Biochem. Z. **258**, 347 (1938).

THE ACID-BASE TITRATION OF PROTEINS[1]

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The more important problems which arise in the determination and the interpretation of the acid-base titration curves of soluble proteins are reviewed. Methods of identifying the number and nature of the ionizing groups are considered. An electrostatic theory, first developed by Linderstrøm-Lang, is applied to the description of the shapes of the curves and to the effect of electrolytes on them. Certain deductions from the theory are outlined.

I. INTRODUCTION

The reaction of a protein with a strong acid or base may be described most comprehensively by a curve having the qualities of a dissociation curve. This curve expresses the relation between the pH of a solution of the protein and a quantity, which we will call h, and which is generally described as the amount of acid or base "bound" to the protein. A series of reaction mixtures are prepared, containing a fixed quantity of protein and varying quantities of a strong acid or base. The pH of each mixture is determined with the aid of an appropriate electrode. The acid or base bound to the protein is computed by subtracting the amount of free hydrogen ion or hydroxide ion in each solution from the amount of acid or base which had been added. We will adopt the convention that h shall be a positive quantity when acid is bound and negative when base is combined. It is the purpose of this paper to discuss the type of information which may be derived from a study of the dissociation curves of soluble proteins and of the effect on them of a number of variables. Some preliminary remarks on the question of standard methods of recording the basic data may be appropriate.

II. THE pH SCALE

The limitations of the pH scale as a scale for the measurement of the relative acidities of acid-base systems are well known. However, there is available no other type of measurement which is comparable in scope and flexibility. In the study of proteins we certainly cannot yet dispense with the pH scale if we are to attempt any consistent correlation of the rapidly accumulating mass of physical and chemical observations on protein solutions. It is proper, therefore, that the reactions of proteins with acids and bases should be described in terms of pH. It is desirable, however, that the scale adopted should be in as close accord with thermodynamic quantities as may be conveniently realizable.

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Two different methods have been advocated for the standardization of the hydrogen-calomel cell. Both seek to establish equality in the ideal relation, pH = $-\log a_{H^+}$, by the assignment of an appropriate value to the potential of the reference electrode. In the simpler method, liquid-junction potentials are assumed to be constant and are implicitly incorporated in the standard potential of the reference electrode. The latter is so chosen as to give, for a standard buffer solution of a weak acid, a pH consistent with the thermodynamic dissociation constant of the acid. MacInnes (19) and others (13) have recently discussed the values of pH which should be assigned to several popular buffer standards. Their recommendations are, undoubtedly, satisfactory when undertaking pH measurements of solutions of weak electrolytes comparable in concentration and in electrolyte pattern with the buffer solution which was chosen as the standard. They admittedly fail in the presence of important concentrations of hydrogen ion and hydroxide ion and, in general, at high ionic strength. In such situations a more laborious procedure has theoretical advantages. In this method, explicit allowance is made for the difference between the potentials of the liquid junction at the standard buffer and at the unknown solution. This is done by subtracting, from the observed potentials of the cell, liquid-junction potentials calculated with the aid of Henderson's equation. The limitations of this equation are admitted. Because this correction is made, the values which are assigned to the standard buffer solutions (11) differ somewhat from those which should be used when liquid-junction potentials are ignored. This method has found favor in studies involving the comparison of pH measurements made at a wide range of ionic strengths. However, there are definite advantages in the adoption of a common practice by all observers. It is suggested, therefore, that all pH measurements should be computed and recorded on the basis of an approved standard, with neglect of liquid-junction potentials. Explicit correction for the latter may then be made by the observer for his own purposes. In the meantime, until a common standard shall have become general, it is desirable that the method adopted in precise studies should always be stated and that the method of establishing the liquid junction should also be given. The purity of protein preparations and the reproducibility of the potentials of their solutions are such as to justify this refinement of definition.

III. THE ISOIONIC POINT

The logical point of origin of the scale of h values is the isoionic point (pI_i), as defined by Sørensen, Linderstrøm-Lang, and Lund (29), rather than the isoelectric point (pI_e) derived from measurements of electrophoretic mobilities or of membrane potentials. The isoelectric point corresponds with the pH at which the net charge is zero, i.e., the number of positive charges on the protein molecule is equal to the number of negative charges. The isoionic point, on the other hand, is the pH at which the number of protons combined on the basic groups is equal to the number dissociated from the acidic groups.[2] The isoelectric and isoionic points are identical only if the protein combines[3] with no ions other than hydrogen ion. In many cases, the value of $\mathrm{p}I_i$ may readily be determined without ambiguity. Proteins, such as the globulins, which are prepared by spontaneous precipitation from substantially salt-free solutions probably contain insignificant amounts of non-protein ions. The pH of a solution of such a preparation will be practically identical with $\mathrm{p}I_i$, provided (*a*) the latter falls within the approximate limits of pH 4.5 and 9.5, (*b*) the concentration of protein is not less than 1 per cent, and (*c*) the concentration of salt is low. If $\mathrm{p}I_i$ has more extreme values than those indicated, it may be determined in the following way: A series of dilute solutions of a strong acid or base are prepared and their pH values are determined. A solution of the pure protein is added to each and the pH is redetermined. Observations are continued until a solution is found the pH of which is unaffected by addition of the protein. This pH may be identified with the isoionic point, since it may be assumed that there has been no combination of the protein with the anion of the acid, or with the cation of the base, at the very low concentrations in which these are present.

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[2] The word "groups" is here understood to refer to uncharged groups such as COOH, NH_2 etc.

Proteins, such as the albumins, which are prepared by salting-out procedures may be far removed from the isoionic condition. Solutions of these preparations often retain some diffusible ions even after protracted dialysis. In this situation one may resort to electrodialysis or may adopt the method of Sørensen (29) and of Sandor (26). The solution of the protein—assuming that $\mathrm{p}I_i$ is below 7—is dialyzed against a dilute solution of ammonia until all diffusible anions have been removed and all cations have presumably been replaced by ammonium ion. The quantity of the latter is determined analytically, an equivalent of strong acid is added, and the pH of the solution is determined. This is identified with $\mathrm{p}I_i$.

Isoionic points may also be located on the dissociation curves by comparing the curves for different concentrations of protein. Provided the solutions contain few diffusible ions, the slopes of the curves will vary with the concentration of protein but will all intersect at $\mathrm{p}I_i$ (*cf.* table 3). It has been suggested that the isoionic point should also be independent of salt concentration. This view received much support from the observation (29) that the curves of egg albumin in solutions of ammonium chloride (0.05–3 *M*) all intersected at $\mathrm{p}I_i$. Unfortunately the situation is not general. It does seem to be true that $\mathrm{p}I_i$ is less sensitive than $\mathrm{p}I_e$ to changes in salt content, but definite effects have been observed in some cases (4) at concentrations of potassium chloride and of other salts exceeding 0.1 *M*. The change in apparent isoionic point depends on the nature both of the salt and of the protein.

IV. THE CALCULATION OF *h*

When *h* is measured from $\mathrm{p}I_i$, it represents the difference between the amount of acid or base which must be added to a protein solution to bring it from $\mathrm{p}I_i$

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[3] The anions or cations which establish electrical neutrality with the protein ions are sometimes loosely described as "bound" to the protein. This, of course, is not to be confused with the binding referred to here, as a result of which the charge on the protein is modified in proportion to the number of ions bound.

to the final pH and the amount of free hydrogen ion or hydroxide ion appearing in the process. The latter is usually determined by measuring the amount of acid or base which must be added to the same quantity of protein-free solvent to effect the same change in pH. The protein-free solution should simulate the electrolyte content of the solution of protein as closely as possible. This procedure involves the assumption that the protein ions do not significantly affect γ_{H^+} or the liquid-junction potential. Errors due to failure of these assumptions are likely to be smaller in solutions containing moderate amounts of a neutral uni-univalent electrolyte than in those which contain protein and acid or base alone. Over the greater part of the dissociation curve, $[H^+]$ and $[OH^-]$ are small, relative to the acid or base added, and small errors in the values assigned to them do not significantly affect the absolute values of h. At extremes of pH, on the other hand, h becomes a small difference between two large quantities and cannot be evaluated accurately. This is one of the considerations which fix the dependable limits of titration of dilute solutions of proteins at about pH 1.5 and 12. Unfortunately, maximum acid- and base-binding capacities of proteins are attained only in these limiting regions. It has been our experience that the most reproducible observations at the extremities of the curves are obtained with solutions containing high concentrations of both protein and potassium chloride.

Values of h are usually recorded in equivalents per gram of protein, although it may sometimes be appropriate to express them in terms of some acceptable molecular weight of the protein. Now, experimental values of the weight of dry protein and of protein nitrogen in a solution are known to depend, in some degree, upon the method of determination. It is important, therefore, that the method of determining the protein content of solutions should be stated.

V. THE NATURE OF THE REACTION OF PROTEIN WITH ACID AND BASE

Irreversible reactions

All proteins are irreversibly affected by a sufficient concentration of hydrogen ion or of hydroxide ion. Some proteins, such as hemoglobin, are denatured rapidly at pH values not far removed from the isoionic point. Others appear to react reversibly with acid or base over a range of pH which may extend from 1.5 to 11 or 12. Denaturation by acid or alkali is generally associated with the formation of a product—a metaprotein—insoluble in the isoelectric region, but the chemical changes involved are not known. They probably vary from protein to protein. In some cases there is evidence of a change in the number of acid- and base-binding groups. Now, some denaturation is to be anticipated at the extremes of pH at which maximum combining capacities are measured. It is evident that the results obtained cannot be taken to correspond with the numbers of ionizing groups in the original protein, unless irreversible reactions with hydrogen ion or hydroxide ion have been excluded.

As evidence of the reversibility of the reactions with acid and base, it has often been held sufficient to demonstrate that the potentials observed showed no consistent drifts with time. It is advisable also to show that a solution, after submission to an extreme pH, retraces an unmodified curve when titrated back to pI_i. This serves, also, to disclose the presence of any metaprotein. In critical situations it may be desirable to show that the protein has suffered no change in some characteristic physical or biological property. On the other hand, such tests may be unduly severe. Many changes in the physical and biological characteristics of proteins are accompanied by no demonstrable change in amphoteric properties.

Too much confidence should not be placed in the absence of drifting potentials. In well-buffered regions significant changes in dissociation may not reveal themselves in noticeable drifts. Moreover, the rate of denaturation may vary with a high power of [H^+] or [OH^-]. In such case, a small addition of acid or base may be sufficient to carry the solution from a pH at which the rate is very small to one at which the reaction is complete in the period allowed for establishment of equilibrium at the electrode. It would, then, only be by chance that a reaction mixture would be encountered in which the irreversible reaction was proceeding at such a rate as to give a persistent drift of potential. In a study (25) of the denaturation of crystalline egg albumin by sodium hydroxide at 25°C., it was found that the reaction followed a roughly bimolecular course, the rate of which was proportional to [OH^-]^4. At initial pH values of 12.50, 12.33, 12.02, and 11.84, it required 3 min., 15 min., 4.5 hr., and 20 hr., respectively, to denature 33 per cent of the protein. The pH fell steadily during the reaction. Dissociation curves of solutions of the partially and completely denatured protein showed that the curve, above pH 8, was progressively displaced from that of the native protein as denaturation proceeded. The changes were consistent with a liberation of weak acid groups, but were not due to carbon dioxide or other diffusible acids. It may be added that similar studies of the denaturation of egg albumin in dilute hydrochloric acid led to the conclusion that this reaction was accompanied by little, if any, change in acid-binding capacity.

Reversible reactions

It is customary to assume that the reversible reaction of proteins with acids and bases is entirely a process of proton transfer. If so, we may expect *h* to be identical with the net charge and pI_i with pI_e. There are, however, well-established cases in which the isoelectric and isoionic points differ materially from one another. In some cases pI_e has been shown to be very sensitive to ionic strength and to the nature of the ions present (32). These effects may be interpreted either as the result of a combination of the protein with specific ions or on certain rather elusive assumptions respecting the individual activity coefficients of the variously charged protein ions which constitute the isoionic assembly of ions (28). Observations of the osmotic pressures, membrane potentials (1), and silver chloride electrode potentials (12) of protein solutions have also been held to be consistent with the specific combination of protein with simple ions[4] such as Cl^-, $C_2H_3O_2^-$, and $H_2PO_4^-$. If such combination does occur, it is presumably an ion association such as that discussed by Bjerrum (3). In so far as the interpretation of dissociation curves is concerned, it will be sufficient, for the present, to acknowledge that combination with ions other than hydrogen ion will affect the relation of *h* to the net charge and, presumably, will modify the slopes of the curves. On the other hand, it will not affect deductions which are based on the identification of stoichiometric points on the curves and which lead to estimates of the number and nature of the ionizing groups in the protein. In the discussion which follows, *h* will be identified with the charge on the protein, leaving open the question whether such anomalies as arise may be ascribed to specific ion combination.

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[4] Striking specific anion effects have been observed in the reaction of insoluble proteins with acids (30).

VI. CHARACTERISTICS OF THE DISSOCIATION CURVES OF PROTEINS

Many examples of dissociation curves of proteins may be found in the literature (6). The proteins the curves of which have been studied most intensively are gelatin, casein, edestin, hemoglobin, egg albumin, β-lactoglobulin, and clupein. Clupein (24) is a very interesting but atypical protein. The work on hemoglobin is distinctive, not only as a contribution to the chemistry of the reaction of hemoglobin with oxygen (35), but also because it included the first careful study of the effect of temperature on the dissociation curve of a protein (34). The investigations on gelatin, casein, and edestin are notable for the fact that the acid-combining capacities were checked by observations employing a cell without liquid junction (12). The properties of many of the proteins mentioned make them unsuitable for investigation over the wide range of conditions to which the method of titration is adaptable. Egg albumin (4) and β-lactoglobulin (5) have proved to be more satisfactory proteins for a study of the characteristics of dissociation curves. They are both reproducible crystalline products, the solutions of which exhibit some, at least, of the criteria of molecular homogeneity. They are soluble over the whole experimental pH range and appear to react reversibly over the greater part of it. Many of their physical properties have been investigated as a function of pH—in particular, their electrophoretic mobilities. Since the latter are, so obviously, a function of charge, the relation of mobility to *h* is of great interest (5, 18).

The curves of individual proteins have much in common. Indeed, they differ only in the relative slope and span of specific regions of the curves. These differences are believed to reflect differences in the relative numbers of certain types of ionizing groups in the individual proteins. The curves in figure 1 will serve to illustrate some of the characteristics which will be discussed in this paper. The very considerable effect of [KCl] on the slope of the curve, which is seen in the figure, will be discussed in a later section. In the main, the change in slope seems to be a function of ionic strength, although small specific ion effects have been observed (4). Two well-defined inflexions are evident in the curves of figure 1. Their positions on the *h*-axis are independent of μ. One occurs at low pH and corresponds to the establishment of a definite maximum cation charge (maximum acid-binding capacity). A second is observed in the neighborhood of pH 8.5. The curves of some proteins are almost horizontal in this region.

In others there is no clear end point, but the value of h at the point of minimum slope can still be determined with fair precision. There have been many attempts to establish experimental values for the maximum anion charge at high pH. The curves of β-lactoglobulin and of some other proteins do appear to be approaching an end point somewhere beyond pH 12. However, they certainly do not attain it within the zone of reversibility of the curves, nor, indeed, at a pH at which dependable values of h may be computed. We are doubtful if measurements of the maximum base bound by proteins may be accepted with the same confidence as those of the maximum acid-combining capacities.

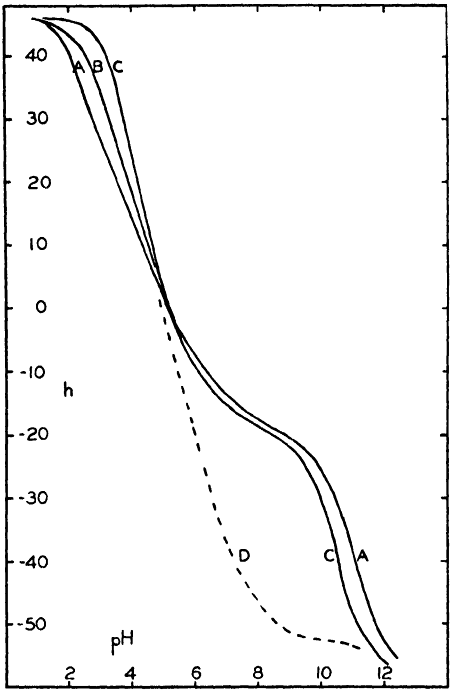

FIG. 1. Dissociation curves of β-lactoglobulin. Curve A, 0.019 M potassium chloride, 0.5 per cent protein; curve B, 0.135 M potassium chloride, 0.5 per cent protein; curve C, 0.67 M potassium chloride, 0.5 per cent protein; curve D, 1 M formaldehyde, 2 per cent protein.

The stoichiometric point near pH 8.5 has been confirmed and a new one, close to pH 6, has been revealed in studies of the effects of temperature on dissociation curves. Wyman (34) found that the displacement with temperature, $\Delta pH/\Delta t$, of the curve of hemoglobin was discontinuous. The curve relating $\Delta pH/\Delta t$ to pH_{25° showed a sharp inflexion close to pH 8.5 and another near pH 5.5. A similar situation has been observed in the curves of all other proteins the temperature coefficients of which have been examined. In the curves of cytochrome c (31) the inflexions appear at about the same levels as in hemoglobin. In β-lactoglobulin, hen albumin, and duck albumin the lower point is close to pH 6.5.

When the titrations of a protein are performed in solutions containing formaldehyde (1–8 per cent), the shape of the curve is greatly modified (figure 1). Above pH 5, the values of $-h$ rise progressively above those found in the absence of the aldehyde, until the curve attains a maximum at about pH 8.5. Thereafter it may remain horizontal up to pH 11, which is the feasible limit of titration in the presence of formaldehyde. An analysis of the curves has shown (14) that the formaldehyde has displaced a definite segment of the curve from the region of pH 10 to about pH 7. This segment is believed to correspond with the free amino groups of the protein.

Other devices for isolating definite segments of a curve have been useful in special cases. The maximum cation charge of a prolamine has been determined by titration in 80 per cent alcohol (8). Titrations of iodized (21) and of deaminized (10) proteins have also been illuminating.

VII. THE ACIDIC AND BASIC GROUPS OF PROTEINS

In table 1 are listed the acidic and basic groups the presence of which in the protein molecule may be anticipated from our knowledge of the composition and structure of proteins. The majority of these groups are those which occur in the side chains of the trivalent amino acids, the names of which are given in the table. In addition to these, such α-amino and α-carboxyl groups as are not combined in peptide or amide linkage will contribute to the dissociation of the protein. In a simple polypeptide, one pair of alpha groups will remain free. If, however, the polypeptide contains cystine, additional alpha groups will be present. Their number will depend on whether the disulfide links occur in a single peptide chain or as cross linkages between two or more chains. In general, any departure from a simple peptide structure may liberate additional alpha groups. Finally, the possible presence of ionizing groups in the non-protein constituent of a complex protein must always be considered. Estimates of the values of the logarithm of the intrinsic dissociation constant, pK_0', and of the temperature coefficient of this constant, $\Delta pK_0'$ (Δt = 10°C.), are included in table 1. These are based upon the dissociation constants (7) and heats of dissociation (34) of simple molecules containing the group to which they refer. The intrinsic dissociation constant of a group may be taken to represent the constant of an isolated representative of that group in the absence of interaction with other charges on the molecule.

Let us assume that the magnitudes quoted in table 1 do represent, in a general way, the acid–base characteristics of the ionizing groups of proteins. The dissociation of a protein may, then, be expected to be concentrated in three zones centered about the pH values 4, 7, and 10–12, respectively, and significant changes in $\Delta pH/\Delta t$ should be observed on passing from one zone to the next. The discontinuities in the temperature coefficients of the experimental curves are entirely consistent with these anticipations, and the sharpness with which $\Delta pH/\Delta t$ has been observed to change in the regions of the two transition points suggests that there is no serious overlapping of adjacent zones. The actual experimental values of $\Delta pH/\Delta t$ indicate that the segment of the curve which lies below pH 6 must be due almost entirely to the dissociation of carboxyl groups, the segment between pH 6 and 8.5 chiefly to that of imidazole groups, and the arm of the curve immediately above pH 8.5 to that of amino groups. Theoretically, α-amino groups should also contribute to the central region of the curve, and the alkaline segment should be a composite of the contributions of the amino, guanidine, phenolic, and sulfhydryl groups. The guanidine groups, however, are so strongly basic[5] that they should exist entirely as cations below pH 11. Their only effect on the curve will be the addition of a fixed positive charge to the net charge which is measured by *h*. There is evidence, also, that the phenolic groups of proteins do not dissociate much below pH 11. The dissociation curves of several proteins containing considerable amounts of tyrosine have been determined in the presence of formaldehyde. They are almost horizontal from pH 8.5 to 11. That is to say, no buffering remains in this region after that of the amino groups has been removed by reaction with formaldehyde. Since phenolic groups are unlikely to be completely dissociated below pH 8.5, we may conclude that they contribute nothing to the curve or to the charge below pH 11 (*cf.* 21). The situation with respect to sulfhydryl groups is obscure. It is probable that most soluble proteins contain few, if any, active SH groups. When they do occur it is likely that they will be found to behave as weak acids similar in strength to the phenolic groups. This leaves only the carboxyl, amino, and imidazole groups to determine the whole sweep of the curve between pH 2 and pH 11. The span of the curve from the end point at low pH to the first transition point close to pH 6 should correspond with the number of carboxyl groups, and the span between this point and the point of inflexion near pH 8.5 with the number of imidazole groups[6]. The number of amino groups can also be deter-

TABLE 1

Ionizing groups of proteins

GROUP	AMINO ACID	pK'_0	Q'	$\Delta pK'$ ($\Delta t = 10°$)
			calories	
α-Carboxyl		3.5	0	0
Carboxyl	Aspartic acid Glutamic acid	4.0	0	0
Imidazole	Histidine	7.0	7,000	−0.17
α-Amino		8.0	10,500	−0.26
Amino	Lysine	10.0	10,500	−0.26
Phenolic	Tyrosine	10.0	6,000	−0.15
Sulfhydryl	Cysteine	10.0	?	?
Guanidine	Arginine	12.5	12,500	−0.31

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[5] Simms (27) has suggested that arginine may be present in some proteins as a cyclic structure—prearginine—in which the guanidine group is replaced by a weakly basic group. The suggestion was made in order to reconcile the dissociation curves of edestin and of gelatin with analyses of the amino acids which they contained. No chemical evidence for the existence of such a structure has yet appeared.

[6] Some justification will be given later for ignoring the possibility that α-amino groups may contribute to the imidazole span.

mined with the aid of dissociation curves of the protein in solutions of formaldehyde. Since the sum of the amino, imidazole, and guanidine groups should be equal to the maximum cation charge, the number of guanidine groups can be obtained by difference.

The significance of a series of differential titrations, such as those which have been outlined, depends upon the precision with which the several end points can be located on the *h*-axis of the curves. Expressed in terms of a protein with a molecular weight of about 40,000, we believe that the probable error in fixing a particular point should not exceed 1 equivalent. Proteins of this size may contain more than 100 ionizing groups and, consequently, the over-all error is small. The error, however, is greatly magnified if it is concentrated in the estimate of a group, such as the imidazole group, which is usually present in proteins in small amount.

There have been many attempts to interpret the curves of proteins in terms of their constituent amino acids (6, 21, 22). The majority of these have been content to show that there is a similarity between the total basic amino acids present and the maximum acid-combining capacity. The more detailed analysis described above has, as yet, been applied only to egg albumin and to β-lactoglobulin. In both cases there is quite satisfactory agreement between the numbers of imidazole and guanidine groups in the intact protein and the moles of histidine and of arginine, respectively, which have been isolated from hydrolysates of the proteins. The numbers of titratable amino groups, however, exceed by 20 to 50 per cent the amounts of lysine isolated from the proteins. Moreover, the titratable carboxyl groups of egg albumin also exceed by 50 per cent the groups which would correspond[7] with the amounts of the dicarboxylic acids which have been isolated. No determinations of the dicarboxylic acids of β-lactoglobulin are available for comparison with the titrations. In considering these discrepancies it is significant that the amino groups measured by titration have been found to be equal to the groups in the intact protein which yield nitrogen with nitrous acid. Furthermore, we have recently obtained evidence (15) that the dicarboxylic acids present in a crude fraction of the hydrolysates of pure egg albumin greatly exceed the amounts of these acids which have been isolated from the hydrolyzed protein. Indeed, the amounts which are present in this fraction are almost sufficient to account for the titratable carboxyl groups of the native protein. It is our belief that the discrepancies which have been referred to are due, in the main, to incomplete isolation of the acidic and basic amino acids. Theorell and Åkesson (31) have suggested that the excess of free amino groups may represent α-amino groups. This may be the situation in some cases, but we doubt if more than one or two of these groups are present in egg albumin or in β-lactoglobulin. Typical α-amino groups should make their contribution to the dissociation curve close to pH 8. This is precisely the region which, in many proteins, is almost horizontal. When, moreover, imidazole groups are measured by titration between the two transition points, the major proportion of the α-amino groups should be included and the titration should exceed the histidine content correspondingly. This discrepancy has not been observed. The alternative is to assume that such α-amino groups as are present are atypical in that they do not contribute to the curve below pH 9. In that case there is no way in which they may be distinguished from the amino groups of the lysine side chains. Dicarboxylic and diamino acids other than those listed in table 1—e.g., hydroxyglutamic acid and hydroxylysine (33)—may be partly responsible for the discrepancies to which we have drawn attention. However, the evidence available at present suggests that the distribution of these amino acids in proteins is limited.

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[7] It is assumed that the free carboxyl groups are equal to the difference between the total dicarboxylic acids and the amide groups.

VIII. THEORETICAL DISSOCIATION CURVES

The dissociation curve of a polyvalent ampholyte can, as von Muralt (20) has pointed out, be simulated by that of an appropriate mixture of univalent acids. A number of reconstructions of protein curves have been made on this principle (7, 9). There is, however, considerable latitude in the choice of the number of different univalent acids which shall be employed, the proportions in which they shall be mixed, and the dissociation constants which shall be assigned to them. The choices made in a successful reconstruction, therefore, bear no necessary relation to the ionizing groups in the protein. Moreover, this method of analysis affords no rational basis for a description of the important effect of ionic strength on the shape of the curve. A more promising approach is that first outlined by Linderstrøm-Lang (17). He derived equations for a hypothetical polyvalent ampholyte which were particularly designed to describe the effect of μ on the dissociation of a protein. Unfortunately, data adequate for a comprehensive test of the usefulness of the theory were not available at the time that it was developed. Quite recently the argument has been adapted and applied to the dissociation curves of egg albumin and of β-lactoglobulin (4, 5) and some aspects of the theory have been reëxamined by Kirkwood (16).

Linderstrøm-Lang based his treatment on Bjerrum's theory (2) of electrostatic interaction in the dissociation of polyvalent acids and on the Debye–Hückel theory of interaction with the ion atmosphere. In the application of these theories to proteins, some simplifying assumptions are made. These are as follows: (*a*) that the protein molecule may be regarded as a sphere, of radius r, on the surface of which there is a random distribution of the charged groups; (*b*) that all groups of a kind may be described by a single intrinsic dissociation constant, K_0; and (*c*) that the several values of K_0, corresponding to the several kinds of ionizing groups, are such that the dissociations of the different kinds of groups do not significantly overlap.

In Kirkwood's treatment the latter assumption is not made. It is, however, a condition which seems to be approximately met in the dissociation of proteins and will be adopted in the discussion which follows. Its adoption allows the dissociation of each kind of group to be treated separately, as that of a symmetrical polyvalent acid. The separate curves may then be combined in a single curve which will deviate only slightly from the theoretical curve in those narrow regions in which significant overlapping occurs.

Let there be m acid groups of the "A" kind in a protein molecule and let the charge on the molecule be $(n - x)$, when x protons have dissociated from the "A" groups. The dissociation of the m protons may be described by m constants (K'), which are related to the m dissociation constants (K) as follows:

$$K'_x = a_{\mathrm{H}^+} \cdot [\mathrm{P}_{n-x}]/[\mathrm{P}_{n-x+1}], = K_x \cdot \gamma_{n-x+1}/\gamma_{n-x} \tag{1}$$

where [P] represents the concentration and γ the activity coefficient of the protein ion the charge of which is denoted by the subscript.

On the basis of Bjerrum's theory of the dissociation of polyvalent acids, we may define K_x in terms of K_0 and a quantity $b = e^2/2\epsilon rkT$, in which e is the charge on the electron, ϵ is the dielectric constant, k is the molecular gas constant, and T is the absolute temperature. The exact relation depends on whether the acid groups of the "A" kind are uncharged or are positively charged. For an uncharged acid group, e.g., COOH, we have

$$K_x = K_0 \cdot e^{2b(n-x+1)} \cdot (m - x + 1)/x \tag{2}$$

and for a positively charged acid, e.g., NH_3^+,

$$K_x = K_0 \cdot e^{2b(n-x)} \cdot (m - x + 1)/x \tag{3}$$

The Debye–Hückel expression for the activity coefficient of a polyvalent ion is now introduced to provide a relation between K_x and K_x. We have

$$- \ln \gamma_1 = \frac{e^2}{2\epsilon kT} \cdot \frac{\kappa}{1 + \kappa a} \tag{4}$$

$$\gamma_{n-x} = e^{-(n-x)^2 \ln \gamma_1} \quad \text{and} \quad \frac{\gamma_{n-x+1}}{\gamma_{n-x}} = e^{2(n-x+0.5) \ln \gamma_1} \tag{5}$$

κ being the thickness of the ion atmosphere and a the distance of closest approach of the ions of the atmosphere to the protein ion. Introducing equation 5 into equation 1 and combining with equations 2 and 3, we have

$$K'_x = K_0^0 \cdot e^{2w(n-x+0.5)} \cdot (m - x + 1)/x \tag{6}$$

which, on conversion to common logarithms, becomes

$$\mathrm{p}K'_x = \mathrm{p}K_0^0 - 0.868w(n - x + 0.5) - \log \frac{m - x + 1}{x} \tag{6a}$$

In equations 6 and 6a,

$$K_0^0 = K_0 e^{\pm b}$$

$$w = b + \ln \gamma_1 = b\left[1 - \frac{\kappa r}{1 + \kappa a}\right] \tag{7}$$

The sign of the exponent in equation 7 is *plus* for the case of an uncharged acid group, and *minus* for the case of a positively charged acid group.[8]

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[8] In the paper on egg albumin (4) this distinction was not made; consequently, the k_0 of that paper, in the case on carboxyl groups, differs from the K_0 defined above. On the other hand, k'_0 and K_0^0 are identical constants.

The m constants defined by equation 6a are symmetrically distributed about their mean, which is identical with $\mathrm{p}K'_{\frac{m+1}{2}}$ and with the mid-point of the dissociation curve ($\mathrm{pH}_{\mathrm{mid.}}$).

$$\sum_{x=1}^{m} \mathrm{p}K'_x/m = \mathrm{p}K^0_0 - 0.868w(n - m/2) = \mathrm{pH}_{\mathrm{mid.}} \tag{8}$$

Let C_p be the bulk concentration of protein. The m equations 1 may be combined to give

$$\frac{[\mathrm{P}_{n-x}]}{C_p} = \frac{K'_1K'_2 \cdots K'_x \cdot a_{\mathrm{H}^+}^{m-x}}{a_{\mathrm{H}^+}^{m} + \sum_{x=1}^{m} K'_1K'_2 \cdots K'_x \cdot a_{\mathrm{H}^+}^{m-x}} \tag{9}$$

and

$$h = \frac{n a_{\mathrm{H}^+}^{m} + \sum_{x=1}^{m} (n - x)K'_1K'_2 \cdots K'_x \cdot a_{\mathrm{H}^+}^{m-x}}{a_{\mathrm{H}^+}^{m} + \sum_{x=1}^{m} K'_1K'_2 \cdots K'_x \cdot a_{\mathrm{H}^+}^{m-x}} \tag{10}$$

When m and n are known, data for the construction of a theoretical curve corresponding to selected values of K^0_0 and w may be computed by introducing equation 6 into equation 10 and solving for h at a suitable range of values of pH. Now the values of w which are compatible with the sizes of protein molecules are quite limited. If we take 20 Å. to represent a minimum radius and set ϵ equal to the dielectric constant of water, then the value of w, at $\mu = 0$, will be about 0.17. As μ increases, w diminishes and approaches zero at high ionic strength. For values of w of this order, the curves are smooth and are practically linear in the region of the mid-point. Linderstrøm-Lang (17) has derived an expression for this limiting slope which may be written as

$$-\Delta\mathrm{pH}/\Delta h = 0.868(w + 2/m) \tag{11}$$

Equation 11 provides a convenient graphic method of obtaining a value for w from any well-defined segment of an experimental curve, i.e., a segment corresponding with a fairly large value of m. The mid-point, $\mathrm{pH}_{\mathrm{mid.}}$, of the segment can be identified with the point at which $h = n - m/2$. By introducing this into equation 8, a value for K^0_0 may be obtained. In this way, all quantities necessary for the solution of equations 6 and 10 are made available, and a comparison can be made of the experimental curve with the theoretical curve which corresponds with these experimental values of K^0_0, w, m, and n. This procedure has been applied to the curves of egg albumin and of β-lactoglobulin. Only the carboxyl regions of the curves are sufficiently broad to permit a full analysis. It has been found that a single value of w does suffice to describe practically the whole span of dissociation of the carboxyl groups at constant μ. Moreover, w has been found to vary with μ in a manner qualitatively consistent with the theory. However, the values of w which best described the curves were all somewhat smaller than the values calculated from equation 7, employing $\epsilon = 78$ and computing r from the diffusion constant of the protein. The ratio of w (theory) to w (found) was about 1.25 for egg albumin and between 1.05 and 1.1 for β-lactoglobulin. So many dubious assumptions are involved in the calculation of w that larger discrepancies than those found might well have been anticipated.

The values of w which were found to describe the dissociation of the carboxyl groups were used to calculate theoretical curves for the imidazole and amino groups of the two proteins. In the case of egg albumin they did fit the experimental curves quite satisfactorily. The theory, however, failed to account for the slopes and positions of the amino segments of the curves of β-lactoglobulin. The values of pK_0 which were found to apply to egg albumin were 4.2 (carboxyl), 6.7 (imidazole), and 10.0 (amino), while those of β-lactoglobulin were 4.5 (carboxyl) and 6.7 (imidazole).

IX. AN APPROXIMATION TO EQUATIONS 6 AND 10

The computations involved in the solution of equation 10 are so tedious that we have sought a simpler relation which would approximate equation 10 for a representative range of values of w and m. Now, when $w = 0$, the curve obtained (*cf.* 20) is identical with that of m equivalents of a univalent acid for which $K = K_0^0$. That is to say, if α represents the fraction of the m groups which have dissociated a proton, then

$$\mathrm{pH} = \mathrm{p}K_0^0 - \log (1 - \alpha)/\alpha \tag{12}$$

A series of curves for a range of positive values of w and a fixed value of m were then constructed from equation 10. When these were compared with the curve for equation 12, it was found that they were displaced from it by an amount approximately equal to $0.868wh$. That is to say,

$$\mathrm{pH} = \mathrm{p}K_0^0 - \log (1 - \alpha)/\alpha - 0.868wh \tag{13}$$

That this relation should hold in the linear segments of the curve is implicit in equation 11. It was encouraging to find that it applied with fair precision over the whole course of a family of curves corresponding to a range of values of w wider than that to be expected in proteins.

At the mid-point ($\mathrm{pH_{mid.}}$) of the curves, $\alpha = 0.5$. Consequently, since $\alpha = (n - h)/m$,

$$\mathrm{pH_{mid.}} = \mathrm{p}K_0^0 - 0.858w(n - 0.5m)$$

and

$$\mathrm{pH} = \mathrm{pH_{mid.}} - \log (1 - \alpha)/\alpha - 0.868wm(\alpha - 0.5) \tag{14}$$

According to equation 14, the slope of the curve relating pH to α should be uniquely determined by the value of the product wm. This offers a simple means of exploring the magnitude of the deviations of the approximate equation 14 from equation 10. The situation is illustrated in table 2, wherein the true values of α at constant wm, but variable m, are compared with the corresponding values, α', calculated from equation 14. It will be seen that α' approximates more and more closely to α as m increases. However, as is seen in the last column, when the deviations are expressed in equivalents rather than in terms of α, they are substantially independent of m. Moreover they exceed 0.1 equivalent at no point on the curves. It is doubtful if the experimental points on the curve of a protein can be reproduced with a smaller probable error than this.

TABLE 2

Comparison of equations 10 and 14

$pK_0^0 = 4.00$; $n = m/2$

$wm = 2.82$

m	4	6	10	20	50	α' (EQUATION 14)	$m(\alpha - \alpha')$
w	0.705	0.470	0.282	0.141	0.0564		
pH	α (EQUATIONS 6 AND 10)						
4.00	0.500	0.500	0.500	0.500	0.500	0.500	
4.3	0.576	0.574	0.573	0.572	0.572	0.571	0.02
4.6	0.651	0.648	0.645	0.643	0.642	0.642	0 04
4.9	0.723	0.718	0.715	0.712	0.710	0.709	0.06
5.2	0.794	0.786	0.781	0.776	0.774	0.773	0.08
5.5	0.852	0.845	0.839	0.835	0.832	0.831	0.08
5.8	0.907	0.897	0.890	0.885	0.882	0.881	0.09
6.1	0.944	0.937	0.930	0.925	0.923	0.921	0.09
6.4	0.970	0.964	0.961	0.957	0.954	0.952	0.08
6.7	0.986	0.981	0.977	0.975	0.973	0.972	0.05

$wm = 1.212$

w	0.303	0.202	0.1212	0.0606	0.0242	α'	$m(d - \alpha')$
pH	α						
4.3	0.614	0.611	0.609	0.607	0.606	0.606	0.03
4.6	0.720	0.715	0.712	0.709	0.707	0.707	0.05
4.9	0.811	0.806	0.802	0.799	0.797	0.796	0.06
5.2	0.885	0.878	0.874	0.870	0.867	0.867	0.07
5.5	0.933	0.929	0.926	0.922	0.921	0.920	0.05
5.8	0.964	0.961	0.958	0.956	0.955	0.954	0.04
6.1	0.983	0.980	0.978	0.976	0.976	0.975	0.03
6.4	0.992	0.990	0.989	0.988	0.987	0.987	0.02

X. SOME DEDUCTIONS FROM THE THEORY

A problem of some interest is that of the effect of molecular size on the dissociation curve of protein. This may be approached by considering how the polymerization of a protein would be reflected in equation 14. The value of m would, obviously, increase in proportion to the increase in molecular weight. On the other hand, w varies with $1/(r + \kappa r^2)$, assuming that $a = r$. Consequently, wm will be proportional to $r^2/(1 + \kappa r)$. In the case of a molecule of the size of the smaller proteins, κr will exceed unity at ionic strengths as low as 0.1. Under these conditions, the limiting relation, in which wm varies with r

(i. e., with $\sqrt[3]{\text{mol. wt.}}$), will be approached. Consequently the span of the curve on the pH-axis should increase with increase in size of the molecule, but large changes in size may be necessary to bring the effect clearly into evidence.

The size of a molecule may also increase through hydration. In this case, since m remains constant, the curve should contract as r increases.

In studies of protein solutions, it is often desirable to estimate the contribution of the protein ions to the ionic strength. A common, but empirical, practice has been to assume that it is equal to one-half the equivalent concentration of the net charge, i.e., to $hC_p/2$. In a protein solution, for example, in which the concentrations of added hydrochloric acid and sodium chloride were [HCl] and [NaCl], we would compute μ from

$$2\mu = [\text{H}^+] + [\text{HCl}] + 2[\text{NaCl}] + h \cdot C_p$$

TABLE 3

The ionic strength of protein ions (β-lactoglobulin)

h	$C_p \times 10^3$, 1.23 [NaCl] $\times 10^3$, 9.0				$C_p \times 10^3$, 0.61 [NaCl] $\times 10^3$, 4.5			
	pH	$\frac{2w}{2.3}$	μ'	μ	pH	$\frac{2w}{2.3}$	μ'	μ
40	2.35	0.033	0.070	0.063	2.24	0.037	0.050	0.035
35	2.78	0.034	0.066	0.054	2.63	0.038	0.045	0.029
30	3.10	0.036	0.055	0.046	2.95	0.041	0.034	0.024
25	3.42	0.038	0.045	0.039	3.25	0.044	0.026	0.021
20	3.70	0.041	0.034	0.033	3.56	0.048	0.018	0.017
15	3.98	0.045	0.024	0.026	3.88	0.052	0.013	0.013
10	4.33	0.048	0.018	0.020	4.25	0.056	0.010	0.010
5	4.74	0.048	0.018	0.014	4.70	0.056	0.010	0.007
0	5.19				5.19			

μ = ionic strength calculated from equation 15.

μ' = ionic strength calculated from w and equation 4.

C_p calculated from molecular weight = 40,000.

Since electrical neutrality requires that [HCl] = [H⁺] + $h \cdot C_p$, this reduces to

$$\mu = [\text{H}^+] + [\text{NaCl}] + h \cdot C_p \tag{15}$$

If [NaCl] is low, μ will increase with h as well as with C_p. We suggest that this may be sufficient explanation for the striking difference in shape between a curve at constant μ and the curve of a protein solution in the substantial absence of salt. In table 3 data from two curves of β-lactoglobulin are recorded. A very small amount of sodium chloride was added to prevent precipitation of the protein at the isoelectric point. With the aid of equation 14 a value of w has been calculated for each point on each curve and the corresponding value of μ has been calculated from equation 7. This is a rather precarious calculation, and the values obtained are to be considered only as coarse approximations. They are compared, in table 3, with values of μ calculated directly from equation 15. It is evident that the effect of the concentration of protein on the dissociation curve is not inconsistent with equation 15.

One final deduction from the theory may be of interest. It touches the question of the nature of the assembly of protein ions in a protein solution. The quantity h represents only the time average of the net charge. It is evident that significant numbers of ions differing materially in charge from h may be present in a given solution. The average concentration of an ion of any particular charge may be calculated from equation 9. To illustrate the situation we have chosen an isoelectric solution of β-lactoglobulin. In figure 2, curves are reproduced giving the distribution of ions, according to equation 9, in this solution at three different ionic strengths.

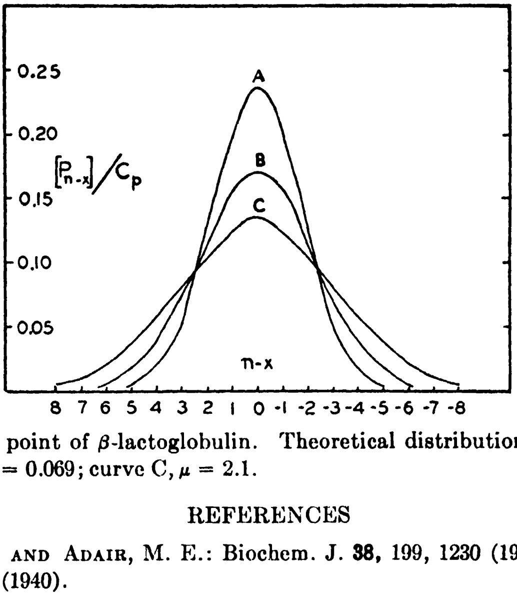

FIG. 2. Isoionic point of β-lactoglobulin. Theoretical distribution of ions. Curve A, $\mu = 0.0$; curve B, $\mu = 0.069$; curve C, $\mu = 2.1$.

REFERENCES

(1) ADAIR, G. A., AND ADAIR, M. E.: Biochem. J. **38**, 199, 1230 (1934); Trans. Faraday Soc. **36**, 23 (1940).
(2) BJERRUM, N.: Z. physik. Chem. **106**, 219 (1923).
(3) BJERRUM, N.: Kgl. Danske Videnskab. Selskab, Math.-fys. Medd. **7**, 9 (1926–27).
(4) CANNAN, R. K., KIBRICK, A. C., AND PALMER, A. H.: Ann. N. Y. Acad. Sci. **41**, 243 (1941).
(5) CANNAN, R. K., PALMER, A. H., AND KIBRICK, A. C.: J. Biol. Chem. **142**, 803 (1942).
(6) COHN, E. J.: Physiol. Rev. **5**, 349 (1935).
(7) COHN, E. J.: Cold Spring Harbor Symposia Quant. Biol. **6**, 8 (1938).
(8) COHN, E. J., EDSALL, J. T., AND BLANCHARD, M. H.: J. Biol. Chem. **105**, 319 (1934).
(9) COHN, E. J., GREEN, A. A., AND BLANCHARD, M. H.: J. Am. Chem. Soc. **59**, 509 (1937).
(10) GREENBERG, D. M., AND SCHMIDT, C. L. A.: Proc. Soc. Exptl. Biol. Med. **21**, 281 (1924).
(11) GUGGENHEIM, E. A., AND SCHINDLER, J. D.: J. Phys. Chem. **38**, 533 (1934).
(12) HITCHCOCK, D. I.: J. Gen. Physiol. **16**, 357 (1932–33).
(13) HITCHCOCK, D. I., AND TAYLOR, A. C.: J. Am. Chem. Soc. **59**, 1812 (1937).
(14) KEKWICK, R. A., AND CANNAN, R. K.: Biochem. J. **30**, 235 (1936).
(15) KIBRICK, A. C.: Unpublished observations.
(16) KIRKWOOD, J. G.: Ann. N. Y. Acad. Sci. **41**, 321 (1941).
(17) LINDERSTRØM-LANG, K.: Compt. rend. trav. lab. Carlsberg **15**, No. 7 (1923–25).

(18) Longsworth, L. G.: Ann. N. Y. Acad. Sci. **41**, 267 (1941).
(19) MacInnes, D. A.: *The Principles of Electrochemistry*, Chap. XV. Reinhold Publishing Corporation, New York (1939).
(20) Muralt, A. L. von: J. Am. Chem. Soc. **52**, 3518 (1930).
(21) Newberger, A.: Biochem. J. **28**, 1982 (1934).
(22) Pauli, W., and Valkó, E.: *Kolloidchemie der Eiweisskörper*. Steinkopff, Dresden (1933).
(23) Pedersen, K. O.: Biochem. J. **30**, 948 (1936).
(24) Rasmussen, K. E., and Linderstrøm-Lang, K.: Compt. rend. trav. lab. Carlsberg **20**, No. 10 (1933-35).
(25) Salk, J., and Cannan, R. K.: Unpublished observations.
(26) Sandor, G.: Bull. soc. chim. biol. **18**, 877 (1936).
(27) Simms, H. S.: J. Gen. Physiol. **14**, 87 (1931).
(28) Smith, E. R. B.: J. Biol. Chem. **113**, 473 (1936).
(29) Sørensen, S. P. L., Linderstrøm-Lang, K., and Lund, E.: Compt. rend. trav. lab. Carlsberg **16**, No. 5 (1926).
(30) Steinhardt, J.: Ann. N. Y. Acad. Sci. **41**, 287 (1941).
(31) Theorell, H., and Åkesson, A.: J. Am. Chem. Soc. **63**, 1818 (1941).
(32) Tiselius, A., and Svensson, H.: Trans. Faraday Soc. **36**, 16 (1940).
(33) Van Slyke, D. D., Hiller, A., and MacFayden, D. A.: J. Biol. Chem. **141**, 681 (1941).
(34) Wyman, J., Jr.: J. Biol. Chem. **127**, 1 (1939).
(35) Wyman, J., Jr.: J. Biol. Chem. **127**, 581 (1939).

SOLUBILITY METHODS IN THE STUDY OF PROTEINS[1]

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Gibbs' phase rule permits one to make certain general predictions about the number of components in a preparation from its solubility curve. If the solubility does not vary with the amount of solid, i.e., it has a constant solubility, the material will in general be a single pure component. If the solubility varies with the amount of solid, two possible cases exist. The material may be a mixture or a solid solution of two or more components.

The curves, methods of distinguishing, and techniques involved in these three cases are discussed in detail.

Use of solubility methods in the purification of proteins is described and illustrated with the cases of pepsin and diphtheria antitoxin.

I. INTRODUCTION

The historical and theoretical development of protein solubility has been thoroughly discussed by Northrop and Kunitz (7, 12) and more recently by Butler (1), so that it is necessary to mention only the more important aspects.

Sørensen (15) seems to have been the first to apply the phase rule to studies of protein solubility. However, when his protein preparations, even after repeated fractionation, did not have a constant solubility as the amount of solid was varied, he suggested (16) that perhaps proteins are dissociable molecules and were for this reason behaving in his solubility experiments as "multiple component systems."

Kunitz and Northrop (7) were the first to obtain highly purified protein preparations of constant solubility. Their work not only demonstrated that proteins could be obtained which had constant solubility and therefore behaved like other molecules of organic or inorganic nature but also proved that not all proteins conformed to the dissociating theory of Sørensen (16). At the present time a number of proteins have been prepared which show a constant solubility, among which are chymotrypsinogen (Kunitz and Northrop (7)), trypsin (Kunitz (5)), swine pepsin (Herriott, Desreux, and Northrop (3)), salmon pepsin (Norris and Elam (10)), ribonuclease (Kunitz (6)), the luteinizing hormone of swine, metakentrin (Shedlovsky, Rothen, Greep, Van Dyke, and Chow (14)), the lactogenic hormones of beef and sheep (Li, Lyons, and Evans (9)), and the oxytocic, pressor, and antidiuretic hormone from beef pituitary (Van Dyke, Greep, Rothen, and Chow (17)).

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II. THE PHASE RULE AND SOLUBILITY CURVES

Crystalline proteins do not have melting points, so that this classical criterion of purity, so useful in organic chemistry, cannot be applied in the study of proteins. However, the somewhat analogous determination of solubility furnishes much the same information.

In most phase-rule studies, changes in certain properties are examined as the temperature or pressure of the system is varied. In the solubility determination the temperature and pressure are held constant, and the variation in the concentration of solute is noted as the amount of solid phase is varied.

The Gibbs' phase rule, which applies only to systems at equilibrium, states that the number of phases plus the number of degrees of freedom equals the number of components plus two, or $P + F = C + 2$. Since the temperature and pressure are held constant during the solubility determination the equation simplifies to $P + F' = C$, in which $F' = F - 2$. In view of the fact that much depends upon the meaning of the terms, the following definitions are taken directly from Findlay's book on the phase rule (2). Thus, "homogeneous physically distinct and mechanically separate portions are called phases." By components are meant "only those constituents which can undergo independent variation in the different phases." For the number of components in a system "there are to be chosen the smallest number of independently variable constituents by means of which the composition of each phase participating in the state of equilibrium can be expressed in the form of a chemical equation." The number of degrees of freedom is "the number of variable factors, temperature, pressure, and concentration of the components, which must be arbitrarily fixed in order that the condition of the system may be perfectly defined."

In order to apply the phase rule to solubility data it is necessary to measure the solubility of the substance in the presence of varying quantities of the solid phase. This is done by stirring varying quantities of the precipitate with a constant volume of solvent until equilibrium is obtained. The concentration of dissolved material is then determined and plotted against the total protein concentration. The various types of curves possible are shown in figure 1.

In the first part of the curve, as small amounts of the unknown solid are added to pure solvent all will dissolve, giving a clear solution, and the points will fall on the 45° line. There is only one phase, the solution, and two or more components one of which is the solvent; therefore there is at least one degree of freedom. As soon as the solid phase appears, there are two phases present. Three types of curves are now possible.

(*1*) A curve with a slope of zero, such as curve A in figure 1 in which the composition of both phases remains constant and independent of the quantity of the phases. This result shows that the system is fixed and has no degrees of freedom. The number of phases and of components is therefore equal. The simplest case is two components, solvent and solute, and two phases, one liquid and one solid. The solid phase consists, therefore, of only one component. It is possible that two solid phases would appear at precisely the same point and the system would then consist of three phases and three components. Such a result could occur if the solid consisted of two proteins present in proportion to their solubility. It corresponds to the case in which a mixture of two substances has the same melting point as one pure substance and is an improbable occurrence. This possibility can be ruled out, however, by repeating the determination in another solvent, since the relative solubility of two substances is, in general, different in different solvents. A racemic mixture of *d*- and *l*-isomers, however, could not be recognized by this method, although if either isomer was present in excess its presence would be detected.

A curve such as curve A in figure 1 with a slope of zero would also be obtained if the solid were a solid solution of two or more proteins having exactly the same solubility. This possibility can also be ruled out by repeating the determination in another solvent.

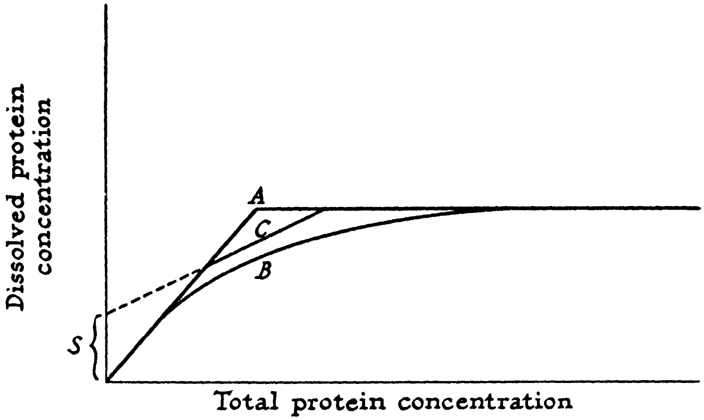

FIG. 1. General types of solubility curves. Curve A, results from a single protein component; curve B, results from a solid solution of two or more protein components; curve C, results from a mixture of two protein components.

(*2*) A rounded curve like curve B of figure 1, in which there is only one solid phase but of varying composition (solid solution of two or more components). This system has at least one degree of freedom.

(*3*) A curve like curve C of figure 1 (a mixture), made up of straight lines in which each break in the curve represents the appearance of a new solid phase.

It may be seen from examination of these three curves that in each case the solubility becomes constant when the excess of solid is great enough. Therefore, it is not sufficient to determine the solubility in the presence of two or more different amounts of solid if these amounts are high relative to the concentration of the dissolved material. One must examine most carefully that region of the curve where the solid phase first appears. If the solubility is the same at this point as in the presence of a large excess of solid, then the preparation has a *constant solubility*.

When it is necessary to distinguish between a mixture and a solid solution with greater certainty than from merely the shape of the curve, this can be done by testing the prediction that the first solid phase that appears along the solubility curve is a pure component if the components are present as a mixture. Thus, if the solid that first appears along the curve is separated off and found to have a constant solubility, the original preparation is a mixture of two or more components. If the solid that is separated off produces a rounded curve similar to the original curve, then the original material is a solid solution. In either case the maximum solubility will be less than that of the original, but the main point is that, in the case of a mixture, the first solid to appear is a single pure component, while in a solid solution it is not.

In the event that one is dealing with a mixture of proteins, certain additional information is obtainable from the solubility diagram (assuming ideal solutions). Thus, if the line obtained when the first solid appears, indicated in figure 1 as C, is extrapolated back, the value at the intersection of this extrapolation with the ordinate is the solubility of the pure component that is appearing as a solid phase. One minus the slope of that line represents the fraction of the pure component in the original suspension. This follows from certain graphic and algebraic considerations which are derived and discussed in the previous papers of Northrop and Kunitz (7, 12) and are not a part of the phase rule.[2]

It may be seen from the preceding discussion that if one obtains a curve of constant solubility similar to curve A in figure 1, the material under examination is either a single component or one of the rare cases of two or more components that has a constant solubility. By determining the solubility in as widely different solvents as the material will permit,—in the case of proteins variations in pH being of particular importance,—one may decide between the possibilities. If, in the different solvents and after all types of fractionation, the material persists as a preparation of constant solubility over the entire range of suspension concentrations, one is forced to conclude that the material is a single pure component or a theoretically possible, though very unlikely, case of a combination of proteins which have the same relative solubility in all solvents used.

III. USE OF THE SOLUBILITY CURVE IN THE PREPARATION OF PROTEINS

Theoretical

One of the most powerful uses that can be made of the solubility curve lies in the development of methods of separating out single protein components. As has already been discussed, in the case of a mixture the first solid to appear is a pure component, so that one has at hand a simple scheme for its preparation.

When the solubility curve is that of a solid solution (curve B of figure 1), the predictions with regard to purifying the components with the same solvent are as follows, assuming that Raoult's law governs at least approximately: (*1*) The greater the difference between the point at which the solid first appears along the curve and the point where the solubility does not change with amount of solid, the greater the difference in the solubility of the pure components and the more suitable the solvent is for separation of the components. (*2*) The solid phase will be richer than the starting material in the less soluble components, especially when most of the total material is dissolved.[3] (*3*) The solution phase will be richer in the more soluble components, particularly when most of the protein is present as the solid phase. (*4*) The maximum separation of components possible for a single treatment will be C times the composition of the starting material, where C is the ratio of the solubilities of the pure components and the composition is expressed in mole fractions. Treating the first fraction a second time will yield a maximum separation of C^2 times the composition of the original preparation, etc. If enough material is available and the difference in solubility is appreciable in the solvent used, relatively pure fractions should be obtainable by repeating the procedure several times. In several instances this has been an experimental fact, as is adequately demonstrated below.

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[2] Kunitz and Northrop (8) have demonstrated with known crystalline proteins how the determined solubility and composition of mixtures and solid solutions agree with the predicted values. They have a case of two proteins which behave as a mixture at one pH and at only 1.5 pH units away form a solid solution.

Purification of pepsin and diphtheria antitoxin

Crystalline pepsin prepared by the method originally described usually has a solubility curve characteristic of a solid solution, as may be seen in figure 2. This preparation had been twice fractionally crystallized and had been found to be strictly homogeneous in the Tiselius electrophoresis cell (4). Similar preparations were homogeneous in the ultracentrifuge (Philpot (13)). Recently a procedure was designed (Herriott, Desreux, and Northrop (3)) for the purification of pepsin on the basis of its being a solid solution of proteins. Starting with the crude commercial pepsin protein, the soluble fraction in the presence of excess solid was saved. The results of the important steps are shown as solubility diagrams in figure 3. The solubility curve of the original crude pepsin is indicated in curve B of figure 3. The solid phase began appearing very early in the curve, as may be seen, and the solubility did not become constant until the suspension concentration was many times greater than that shown on the graph. This is typical of a solid solution curve. The material was then extracted with a volume of solvent in which only a third of the total protein dissolved but, as may be seen from the curve marked "soluble fraction" in figure 3, that portion that was soluble in the extracting solvent contained very little of the less soluble material. The residue from the extraction, on the other hand, was rich in the less soluble component, as is indicated by the solubility curve marked "insoluble fraction." When the soluble fraction was precipitated and reëxtracted in the same solvent as used before, the amount dissolved was considerably greater, as was to be expected. When the soluble portion after this second extraction was crystallized it had a constant solubility, as seen from the solubility curve A of figure 3 and it remained constant on further fractionation.

The case of diphtheria antitoxin (Northrop (11)) is similar to that of pepsin but is worth discussing in order to emphasize that purification may be accomplished and examined by the solubility technique.

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[3] In the paper by Northrop and Kunitz (12) line 8 of the text on page 787 should read, "the first precipitate that appears will be $\frac{C}{F}$" $\left(\text{not } \frac{1}{FC}\right)$.

Figure 4 contains the solubility curves of the fractions of diphtheria antitoxin protein at various stages in the purification. The entire protein fraction, after precipitation of the antitoxin with toxin and then removal of the toxin, had a solubility curve similar to the top curve, A, of figure 4. The fraction of this preparation which was insoluble between 0.1–0.35 saturated ammonium sulfate is shown in curve A. It was a very insoluble material, for the coördinates are on a 10 to 1 scale. The fraction precipitated between 0.35–0.65 saturated ammonium sulfate is shown in curve B. Here the coördinates have the same scale,

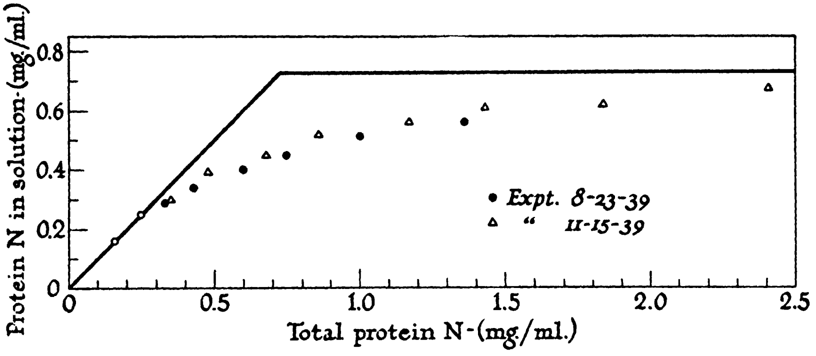

FIG. 2. Solubility curve of twice-crystallized pepsin

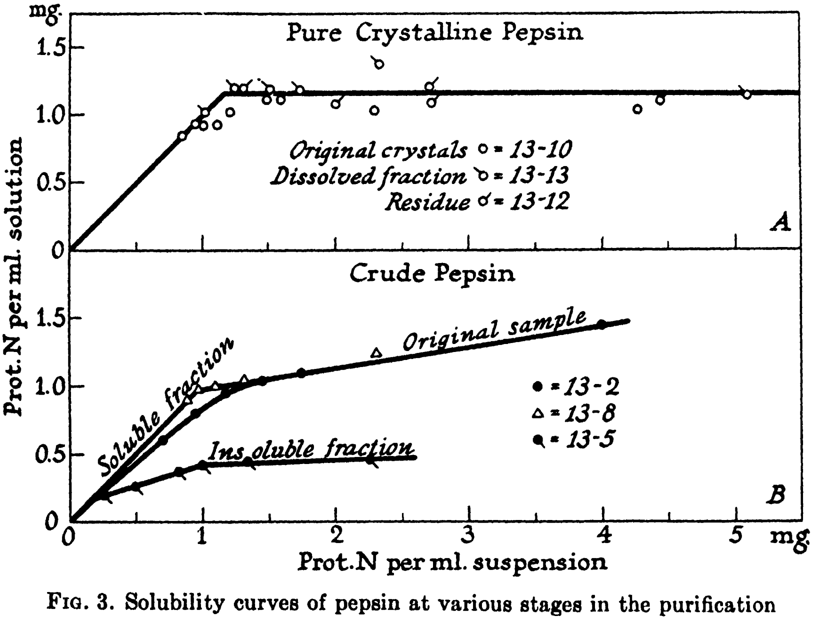

FIG. 3. Solubility curves of pepsin at various stages in the purification

and it is quite clear that this fraction was more soluble but far from homogeneous. When this last fraction was refractionated by saving only that part which was soluble in 0.50 ammonium sulfate but insoluble in 0.65, the curve C was obtained. Upon repeating this fractionation between 0.5–0.65 saturation a material of constant solubility was obtained, as seen by curve D.

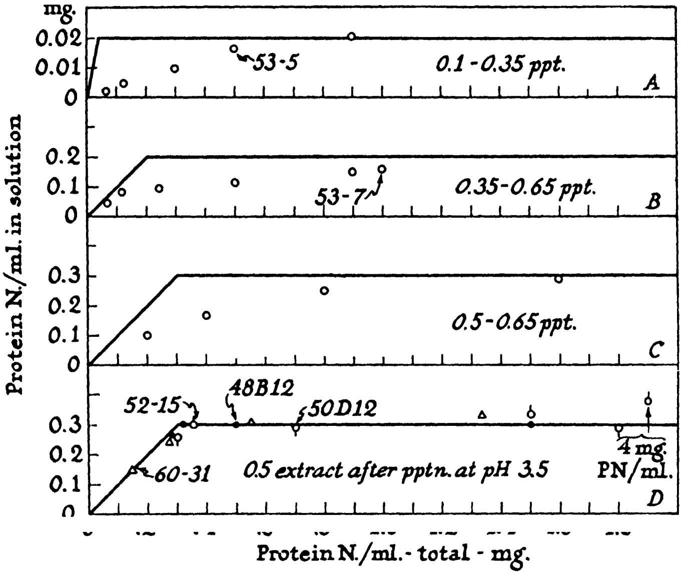

FIG. 4. Solubility curves of diphtheria antitoxin (Northrop) at various stages in the purification.

IV. CONDITIONS AND CERTAIN DETAILS OF SOLUBILITY TECHNIQUES

Solvent.—Although there are few definite rules that apply to all proteins, certain general aspects of the problem must be kept in mind. Thus, it is important when choosing the conditions to arrange them so as to obtain as near the maximum stability of the protein as possible. A solvent of such a nature (usually a salt solution) should be chosen that the amount of dissolved protein will be such as to be estimated precisely and yet not such that too large a proportion of the protein in a concentrated suspension is in solution.

Amorphous and crystalline forms.—With some proteins it is possible to perform the solubility experiment on either the crystalline or the amorphous form. There are certain advantages to each but, other things being equal, a solubility experiment on the crystals is open to less objection. On the other hand, solubility curves of amorphous forms are often extremely convenient and useful, especially for proteins that do not crystallize.

Equilibrium from both sides.—In addition to the choice between solubility curves for amorphous and for crystalline forms, one may choose to approach the equilibrium point from either the supersaturated or the undersaturated side.

Both approaches should be made when the amount of material and other considerations permit, and it is rather important that at least one or two points be approached from both sides to insure one that a true equilibrium value is actually being measured. Approach from the supersaturated side of the equilibrium can be conveniently carried out with amorphous preparations, using a split solvent. This is done by dissolving the solid phase or suspension in the water or buffer part of the solvent and precipitating with the concentrated salt part of the solvent. With crystals, supersaturation is easily obtained by varying the temperature so that some of the crystals dissolve and, on returning to the original temperature, crystallization takes place, but slowly.

Approaching from the undersaturated side of the equilibrium is usually accomplished for both amorphous and crystalline forms by stirring the solid phase with the complete solvent. The time required to attain equilibrium will depend upon a number of factors, paramount among which are the quantity of solid, the size of the solid particles, and the rate of stirring. The time will therefore vary with conditions and must in each case be determined experimentally.

Preliminary equilibration.—In bringing the solid into equilibrium with the solvent before carrying out the solubility-curve experiment, much time can be saved by first washing the solid on a Büchner funnel with small aliquots of the complete solvent or by dialysis in cellophane bags against the solvent. The solid is then stirred or precipitated with successive aliquots of the solvent until several successive aliquots contain the same amount of dissolved protein. If one does not obtain a constant amount dissolved after a few washings it may be due to a change in the protein composition, owing to removal of a relatively large amount of the more soluble components in the washing, and not because salt equilibrium had not been obtained. When several successive aliquots do have the same amount of dissolved protein, it may be presumed that equilibrium exists between the solid and the solvent.

Distribution.—Solvent equilibrium having been obtained, the suspension is then distributed in varying amounts in a number of test tubes, followed by the addition of the solvent. The amount of suspension in each tube is adjusted, so that at equilibrium a range from complete solution of the protein to a large excess of solid exists in the series of tubes. For reasons already discussed in the earlier sections of this paper, it is more important to have most of the tubes in the region of a small excess of solid than of a great excess of solid.

Final equilibration.—In stirring the protein solutions or suspensions, foaming should be avoided as this may bring about considerable denaturation. It has been found that glass beads or marbles in test tubes that are completely filled, stoppered, and then rotated, suffice to stir the suspensions. The time of stirring necessary to attain equilibrium must be determined experimentally.

Separation of solid and solution phases.—After equilibrium has been attained, the solid is separated from the solution by centrifugation or filtration. Care must be taken in the case of "amorphous" solubility curves that are approached from the supersaturated side that equilibrium is attained before separation of the solid phase. It has usually been assumed that one could not have a protein solution supersaturated with respect to the amorphous form, but certain experiments with pepsin seem to indicate that supersaturation is possible. The filtrates should remain clear for several hours unless crystallization is taking place, which can be determined by microscopic examination. In the event the preparation tends to crystallize in the complete solvent, one may depress this tendency by allowing the solution of protein to stand in the dissolving half of the split solvent for several hours before adding the precipitating half of the solvent. This allows the last traces of crystals to dissolve, which would otherwise act as seeding crystals. The supernatant or filtrate must, therefore, be crystal-clear. It has been found that filtration through Whatman's No. 42 filter paper gives uniformly the best results. If the amount of dissolved protein is under 0.5 mg. per milliliter the filter paper takes up some of the protein, so that the first portion of filtrate may be low in dissolved protein and is therefore usually discarded.

Estimation of dissolved protein.—The amount of dissolved protein may be determined by any of a number of methods, such as Kjeldahl nitrogen, turbidity, biological activity, tyrosine color value, or any other easy and precise method. In the event that one is examining an unidentified substance having a specific property such as a biological activity, one can learn a great deal about the class of substances in which the unknown belongs by making as many different tests on the solubility solutions as possible. For example, one will find that the protein, carbohydrate, nucleic acid, or other properties do or do not run parallel to the specific property that is being studied.

REFERENCES

(1) Butler, J. A. V.: J. Gen. Physiol. **24,** 189 (1940).

(2) Findlay, A.: *The Phase Rule and its Applications.* Longmans, Green and Company, New York (1938).

(3) Herriott, R. M., Desreux, V., and Northrop, J. H.: J. Gen. Physiol. **24,** 213 (1940).

(4) Herriott, R. M., Desreux, V., and Northrop, J. H.: J. Gen Physiol. **23,** 439 (1940).

(5) Kunitz, M.: J. Gen. Physiol. **21,** 601 (1938).

(6) Kunitz, M.: J. Gen. Physiol. **24,** 15 (1940).

(7) Kunitz, M., and Northrop, J. H.: Comp. rend. trav. lab. Carlsberg **22,** 288 (1938).

(8) Kunitz, M., and Northrop, J. H.: Cold Spring Harbor Symposia Quant. Biol. **6,** 325 (1938).

(9) Li, C. H., Lyons, W. R., and Evans, H. M.: J. Biol. Chem. **140,** 43 (1941).

(10) Norris, E. R., and Elam, D. W.: J. Biol. Chem. **134,** 443 (1940).

(11) Northrop, J. H.: Science **93,** 92 (1941); J. Gen. Physiol. **25,** 465 (1941).

(12) Northrop, J. H., and Kunitz, M.: J. Gen. Physiol. **13,** 781 (1930).

(13) Philpot, J. St. L.: Biochem. J. **29,** 2458 (1935).

(14) Shedlovsky, T., Rothen, A., Greep, R. O., Van Dyke, H. B., and Chow, B. F.: Science **92,** 178 (1940).

(15) Sørensen, S. P. L., and Höyrup, M.: Compt. rend. trav. lab. Carlsberg **12,** 213 (1917).

(16) Sørensen, S. P. L.: Compt. rend. trav. lab. Carlsberg **18,** No. 5 (1930).

(17) Van Dyke, H. H., Greep, R. O., Rothen, A., and Chow, B. F.: Unpublished work, 1941–42.

PROTEIN CONSTITUENT ANALYSIS BY THE SOLUBILITY METHOD[1]

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The principles of the solubility-product method and their application to the determination of amino acids in protein hydrolysates are outlined. The methods for leucine and glycine serve as specific examples. The properties of aromatic sulfonic acids as reagents for amino acids are included to illustrate their use in solubility analysis and in the qualitative fractionation of mixtures of amino acids.

I. INTRODUCTION

Protein chemistry requires as a fundamental basis the accurate establishment of the amino acid composition of the various proteins. The problem is analogous in principle to the establishment of the composition of simpler molecules in terms of their constituent atoms. In practice, many diverse approaches have been made to the problem of analysis for amino acid constituents. Yet the relative difficulty of the task is evidenced by the fact that we cannot write today the empirical formula for the amino acid composition of even the simplest protein, and among many of the more complex proteins 40 per cent or more of the amino acids in the molecule remain completely unaccounted for.

Fractionation procedures, precipitation methods, and colorimetric methods have all been applied, with varying degrees of success, to the analysis of complicated mixtures of amino acids. For a few of the amino acids, results of high precision have been attained; for others, however, the accuracy has been less satisfactory, and for many of the constituent parts of the protein molecule there has existed no method which could be classified as a quantitative determination. The results acquired with the aid of the existing quantitative methods, together with the significant contributions of the qualitative methods, have been of immeasurable assistance in the progress of protein chemistry. Nevertheless, the history of this field for the past fifty years seems to point to the conclusion that a major advance in the *quantitative* knowledge of protein structure (complete analysis) can be expected only through the introduction of some fundamentally new approach.

The solubility method represents one attempt at a new solution of this problem (3, 11). Another approach is provided by the isotope dilution method of Rittenberg and Foster (8, 9). The solubility method, which is the subject of this discussion, has originated from work on the gravimetric determination of amino acids and represents an attempt to extend the range of this type of analytical approach.

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[1] Presented at the Symposium on Physicochemical Methods in Protein Chemistry, which was held under the joint auspices of the Division of Physical and Inorganic Chemistry and the Division of Biological Chemistry at the 102nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September 8–12, 1941.

In gravimetric analysis a familiar procedure consists in the precipitation of a substance in the form of a sparingly soluble salt. The solubility product of the ions involved in the equilibrium is the principal factor determining the completeness of precipitation. This feature is, of course, common to inorganic as well as organic analysis,—to the determination of barium as sulfate as well as to the determination of arginine as flavianate. In the amino acid field the difficulty of attaining quantitative isolation is evidenced by the fact that in only a very few instances are reagents known which are capable of quantitatively precipitating one and only one amino acid from a mixture so complex as a protein hydrolysate. A second procedure, which has of necessity been followed in some cases, has been the use of reagents which precipitate only the major part of the amino acid, supplemented by the application of corrections for the amount of amino acid remaining in solution in the hydrolysate. There is no sound method for determining this correction factor, however, since the absolute value of the solubility of a compound is a function of the composition of the solvent. The solubility determined in water or normal hydrochloric acid may be far different from the solubility in a protein hydrolysate. At this point in the problem the solubility-product method was introduced in order to provide a sound principle for the calculation of the total amount of an amino acid in a solution from data obtained by partial precipitation of the amino acid.

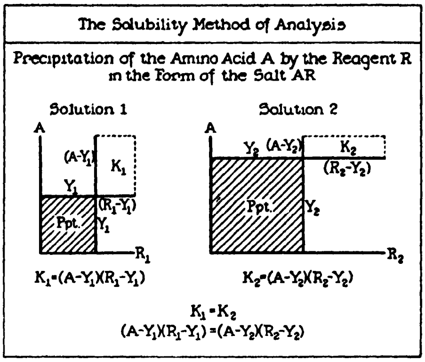

FIG. 1. The solubility-product method applied to the analysis for an amino acid under conditions of partial precipitation.

II. PRINCIPLES OF THE SOLUBILITY-PRODUCT METHOD

The first formulation of the method (3, 11) is illustrated in figure 1. Two aliquots of a solution are taken, each containing A moles of the amino acid to be determined. To the first aliquot is added R_1 moles of a reagent which causes the partial precipitation (50 to 60 per cent) of the amino acid as a salt. If the precipitated salt (Y_1 moles) is binary, containing equimolar quantities of amino acid and reagent, it may be represented by the shaded square in figure 1. Remaining in solution are $A - Y_1$ moles of the amino acid and $R_1 - Y_1$ moles of the reagent.

To a second aliquot is added a larger quantity of reagent, R_2, causing an increased precipitation up to, for example, 70 to 80 per cent of the amino acid present. The method of analysis is founded on the experimental observation that under conditions such as these the solubility product, that is, the product of the molar quantities of the amino acid and the reagent remaining in solution, is practically constant. In this case the solubility product for the first point is $(A - Y_1)(R_1 - Y_1)$, represented by the area K_1, and for the second point $(A - Y_2)(R_2 - Y_2)$, or the area K_2. If K_1 and K_2 have identical, or, as will be shown later, nearly identical values, they can be eliminated by equating the two products, and the resulting expression can be solved for the only unknown, A. This calculation yields the total amount of amino acid present in the sample under analysis.

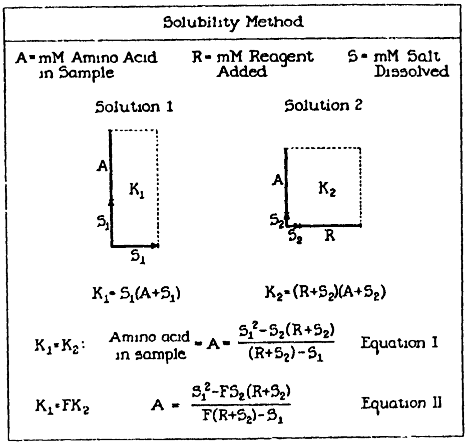
Solubility Method
A = mM Amino Acid in Sample
R = mM Reagent Added
S = mM Salt Dissolved
Solution 1
Solution 2
A
K_1
S_1
S_1
A
K_2
S_2
S_2
R
$K_1 = S_1(A+S_1)$
$K_2 = (R+S_2)(A+S_2)$
$K_1 = K_2$: Amino acid in sample $= A = \frac{S_1^2 - S_2(R+S_2)}{(R+S_2) - S_1}$ Equation I
$K_1 = FK_2$ $A = \frac{S_1^2 - FS_2(R+S_2)}{F(R+S_2) - S_1}$ Equation II

FIG. 2. The solubility method applied under conditions in which the solid phase is preformed and allowed to dissolve *in* the sample instead of being formed by precipitation *from* the sample. The term R represents that portion of the sulfonic acid added as such (or as a sodium salt) to the second solution, in contradistinction to the amount of the same sulfonic acid contributed to the solution by the salt (S) as it dissolves.

By this procedure it is possible to carry out an analysis without quantitative precipitation. The development of the method can be carried still one step further, however, and the precipitation requirement completely eliminated. The solubility-product principle may be applied under conditions in which measurement is made of the amount of an amino acid salt going *into* solution in aliquots of the sample under analysis (2). In this case no isolation of the amino acid from the hydrolysate is required. One might say that the method in this form permits the quantitative determination of an amino acid in solution from measurements of the extent to which its presence depresses the solubility of one of its pure salts. This technique possesses several advantages from the experimental standpoint. The working procedure of the method in its present applications to the analysis of protein hydrolysates is illustrated in figure 2.

The determination of *l*-leucine with 2-bromotoluene-5-sulfonic acid may be discussed as an example of this procedure. An excess of a known pure sample of *l*-leucine 2-bromotoluene-5-sulfonate is added to an aliquot of the hydrolysate containing an unknown quantity (A millimoles) of *l*-leucine, and the salt allowed to dissolve to the point of saturation. The binary salt dissolved (S_1 millimoles) contributes equimolar amounts of *l*-leucine and 2-bromotoluene-5-sulfonic acid to the solution. At equilibrium the total *l*-leucine present is $A + S_1$ and the total sulfonic acid is S_1, giving the solubility product $K_1 = S_1(A + S_1)$. Simultaneously, the *l*-leucine salt is added to a second aliquot of the hydrolysate in which R millimoles of sodium 2-bromotoluene-5-sulfonate has previously been dissolved. At equilibrium a smaller quantity of the amino acid salt (S_2) dissolves in this second solution as a result of the common-ion effect of the added sulfonic acid. The total *l*-leucine in solution is $A + S_2$ and the total sulfonic acid is $R + S_2$, giving the solubility product $K_2 = (R + S_2)(A + S_2)$. If the two solubility products are equal, solving for A gives the amount of *l*-leucine in the sample in terms of the three known quantities S_1, S_2, and R (equation I, figure 2). This equation is applicable to the amino acid determinations in which K is found to be constant. In other determinations, such as that of glycine as nitronaphthalenesulfonate, solubility products of satisfactory constancy are not obtained. In these cases K_2 is consistently greater than K_1. This deviation, however, is small and reproducible, and, as will be demonstrated later, the introduction of a factor F in the equation adequately accounts for this increase (equation II, figure 2).

Experimentally, the method involves the measurement of the solubility of a given solid phase in two aliquots of the solution of unknown amino acid content. This measurement can be made with accuracy gravimetrically. A weighed amount of the amino acid salt is added to a sample of the solution. The solubility is determined by difference after equilibrium has been attained at 0°C. The separation of the two phases without temperature change is accomplished by centrifugation at 0°C. through sintered glass (6). The undissolved amino acid salt, together with a small amount of adhering solution, is retained by the sintered glass. The filter assembly is weighed before and after drying and the loss on drying provides a measure of the adhering solution, making it possible to calculate the true net weight of the solid phase.

The application of the solubility method under these conditions permits the purity of the solid phase to be readily insured. The initial amino acid salt represents a known preparation of established purity. As it dissolves, however, it contributes to the solution a quantity of sulfonic acid which might cause the precipitation of some other salt from the unknown. To establish the absence of interference, the first step is the addition of slightly more than $R + S_2$ millimoles of sulfonic acid to a separate sample of the hydrolysate. This concentration of sulfonic acid should not be sufficient to cause the precipitation of any compound from the unknown, and a clear solution should be obtained. The second and final check is made by adding a small amount of the pure amino acid to the A millimoles present and determining the precision of recovery.

This proof of purity of the solid phase establishes as a corollary principle the *specificity* of the determination. It is obvious that if *l*-leucine is the only amino acid in the solid phase, then it is the only amino acid taking an active part in the equilibrium between solid phase and solution, and the analysis is fundamentally specific for *l*-leucine.

It will be observed that the solubility method does not demand reagents of high selectivity. For example, given a mixture of approximately equimolar quantities of leucine, phenylalanine, and tyrosine, it would theoretically be possible to analyze for each of the three components with the aid of a single reagent. This could be done provided the leucine, phenylalanine, and tyrosine salts had about the same solubility product. For the determination of leucine, the leucine salt would be employed as solid phase; for phenylalanine, the phenylalanine salt; and for tyrosine, the tyrosine salt. This analysis by the solubility method would be possible even though the reagent would not be considered selective for any one of the three amino acids in the usual sense of the word.

TABLE 1

Determinations of l-leucine

Reagent: sodium 2-bromotoluene-5-sulfonate

$$l\text{-Leucine} = \frac{S_1^2 - S_2(R + S_2)}{(R + S_2) - S_1}$$

l-LEUCINE CONTENT PER 3 CC.	ALSO PRESENT PER 3 CC. IN ADDITION TO N HCl	SOLUBILITY PRODUCT[*]		*l*-LEUCINE FOUND	
		K_1	K_2		
mg.				*mg.*	*per cent recovery*
12.00		9.27×10^{-4}	9.33×10^{-4}	11.74	97.8
14.01	10% Methyl cellosolve	12.10	12.07	14.13	100.8
14.90	15% Methyl cellosolve	13.68	13.73	14.77	99.1
12.13	Arginine, 31.8 mg. Glutamic acid, 26.4 mg.	12.38	12.43	11.95	98.5
	Glycine, 13.5 mg. Tyrosine, 32.4 mg.	12.29	12.37	11.88	98.0

[*] K_1 and K_2 have been calculated on the basis of moles per liter.

III. APPLICATION TO THE DETERMINATION OF AMINO ACIDS

A. Leucine

The establishment of the precision of the solubility-product principle for a given amino acid and reagent combination is illustrated for *l*-leucine 2-bromotoluene-5-sulfonate in table 1. The determinations are carried out in hydrochloric acid solution (1.0 ± 0.2 normal). The amount of sodium 2-bromotoluene-5-sulfonate added to the second aliquot in each case is about 0.1 millimole. The agreement between K_1 and K_2 is satisfactory, and equation I gives accurate recoveries for *l*-leucine. In protein hydrolysates, of course, the solubility product will be higher than in a pure leucine solution. When the value of the solubility product is increased from 9×10^{-4} up to 12 or 13×10^{-4} by the addition of an organic solvent, the analysis is equally satisfactory. When the solubility product is similarly increased by the addition of arginine, glutamic acid, glycine, and tyrosine, the accuracy is maintained. An important characteristic of the method is illustrated in these data. As has been pointed out, in contrast to the usual gravimetric techniques, the solubility method is in principle independent of the absolute magnitude of the solubility of the salt in the solvent or unknown hydrolysate. The relative constancy of the two K's in any individual leucine determination is the fundamental essential.

From the stereochemical standpoint, the analysis under these conditions is specific for *l*-leucine, and small amounts of the *d*-isomer, if present, do not enter into the equilibrium. Analysis for *d*-leucine can be made by use of the *d*-salt as solid phase. Analysis for both of the isomers in mixtures of *d*- and *l*-leucine may be accomplished by the use of the racemic mixture *dl*-leucine 2-bromotoluene-5-sulfonate as solid phase. Theoretically, if a *dl*-salt is a racemic *mixture*, not a racemic *compound*, K_{dl} is equal to twice K_l in the ideal case. This relationship holds for the *dl*-leucine salt of this reagent, but does not hold, of course, for all *dl*-amino acid salts, many of which are racemic compounds.

B. Glycine

A series of analyses for glycine, using nitronaphthalenesulfonic acid, is listed in table 2. In the first example, K_1 is 7.33×10^{-4}, and when about 0.1 millimole of nitronaphthalenesulfonic acid has been added, K_2 is 7.83×10^{-4}. If solved by equation I, as used for leucine, this analysis would give a recovery of about 80 per cent, the low value being the result of the small but measurable difference between K_1 and K_2. In the first determination it will be observed that K_1 is 93.6 per cent of K_2. As the magnitude of K_1 is increased over a relatively wide range, namely, from 7×10^{-4} up to 17×10^{-4}, the ratio K_1/K_2 remains satisfactorily constant. This relationship holds also for analyses in the presence of glucose, of furfural, and of arginine, glutamic acid, leucine, and tyrosine. If an average value of 0.939 is taken for the ratio K_1/K_2 and this figure substituted for F in equation II, the recoveries of glycine calculated by this equation all fall within the desired range of precision.

The change in the solubility product occurring in this determination of glycine as nitronaphthalenesulfonate may be considered to represent more closely the general case rather than an exception to the rule. The constancy of K ($K_1/K_2 = 1.00$) in the leucine analysis is an experimental fact. It is known, however, to be the result of compensating factors which in the glycine case fail to compensate as completely. By independent experiments it can be shown that changes in amino acid concentration, sulfonic acid concentration, or sodium chloride content (if the sodium salt of the reagent is used) all exert small but measurable effects on the solubility product. But the addition of a given amount of a sulfonic acid reagent, a factor which can be controlled, produces nearly the same series of concentration changes in each case. It has been shown for the leucine and glycine determinations that the ratio K_1/K_2 can be maintained constant over a range of K_1 values. A constant K_1/K_2 ratio is equally as satisfactory mathematically as a constant solubility product. These effects do impose the requirement that for accurate analysis the concentration of the protein hydrolysate must be adjusted so that the initial amino acid concentration is close to the value used in these experiments. As is true of many analytical methods, the first analysis on an unknown generally serves to indicate the optimum working range for subsequent accurate determination.

TABLE 2

Determinations of glycine

Reagent: 5-nitronaphthalene-1-sulfonic acid

$$\text{Glycine} \quad \frac{S_1^2 - 0.939S_2(R + S_2)}{0.939(R + S_2) - S_1}$$

GLYCINE CONTENT PER 3 CC.	ALSO PRESENT PER 3 CC. IN ADDITION TO N HCl	SOLUBILITY PRODUCT*		$\frac{K_1}{K_2}$	GLYCINE FOUND	
		K_1	K_2			
mg.					*mg.*	*per cent recovery*
6.62	25% Methyl cellosolve	7.33×10^{-4}	7.83×10^{-4}	0.936	6.56	99.1
6.85	30% Methyl cellosolve	9.39	10.06	0.934	6.73	98.2
6.90	35% Methyl cellosolve	12.07	12.82	0.942	6.96	100.8
7.89	40% Methyl cellosolve	17.00	18.10	0.939	7.89	100.0
6.76	30% Methyl cellosolve Glucose, 21.6 mg.	9.23	9.79	0.943	6.83	101.0
6.84	30% Methyl cellosolve Furfural, 26 mg.	10.84	11.50	0.943	6.91	101.0
6.79	30% Methyl cellosolve Arginine, 10.0 mg.	12.14	12.84	0.946	6.97	102.7
	Glutamic acid, 26.4 mg. Leucine, 24.0 mg. Tyrosine, 18.0 mg.	12.19	12.92	0.944	6.94	102.2

* K_1 and K_2 have been calculated on the basis of moles per liter.

TABLE 3

Determination of glycine and l-leucine in a synthetic mixture of amino acids

PERCENTAGE COMPOSITION OF MIXTURE		ANALYSIS FOR GLYCINE		ANALYSIS FOR *l*-LEUCINE	
		Found	Per cent recovery	Found	Per cent recovery
Alanine	5.8				
Arginine	4.8	5.83	99.2		
Aspartic acid	11.5	5.88	100.0		
Cystine	5.8	5.90	100.3		
Glutamic acid	11.5	5.80	98.7		
Glycine	5.88				
Histidine	3.5				
Leucine	11.51				
Lysine	3.8				
Phenylalanine	11.5				
Proline	11.5			11.40	99.1
Tyrosine	11.5			11.57	100.6
Ammonia	1.5			11.41	99.2

The validity of equations I and II for leucine and for glycine has been checked on a number of known mixtures other than those already listed. One example is summarized in table 3. A mixture composed of thirteen constituents, approaching in complexity a protein hydrolysate, has been analyzed with satisfactory precision for both leucine and glycine by the solubility method. The final step is from synthetic mixtures of this type to protein hydrolysates. Two examples may be sufficient to demonstrate the practical application of the method (table 4). In the analysis for glycine and leucine in hydrolysates of silk fibroin and egg albumin, both the reproducibility and the recovery are comparable to that attained with synthetic mixtures. It should be pointed out, however, that experimentally the values in table 4 are based on the amino acid contents of the *hydrolysates*. It is incorrect to conclude that each value necessarily represents the total glycine or leucine content of the protein preparation employed. Before such a conclusion is justified, further information is required on the accuracy of the procedures generally used for the hydrolysis of proteins and (except in the case of glycine) on the extent of racemization during hydrolysis. The solubility method is being employed to investigate both of these problems, each of them being fundamental to the final determination of protein composition.

TABLE 4

Determination of glycine and l-leucine in protein hydrolysates

SILK FIBROIN		EGG ALBUMIN	
	per cent		*per cent*
Glycine	44.0	*l*-Leucine	9.11
	44.6		9.12
	43.2		9.00
	43.3		8.95
	44.4		9.11
	44.6		9.12
	Av. 44.0		Av. 9.07
Recovery on protein hydrolysate plus added glycine	99.5	Recovery on protein hydrolysate plus added *l*-leucine	100.5

Of the amino acids which come within the present scope of the method, leucine and glycine have been singled out for illustration in this discussion. Leucine, with its lack of characteristic functional groups, has previously been indeterminable. Glycine has similarly been a difficult amino acid to estimate, especially when present in relatively small amounts. The method to date has also been applied to phenylalanine, tyrosine, and proline. It may be mentioned that this analytical approach is not limited to amino acids, being in theory applicable to the determination of any substance possessing an acidic or basic group which forms sparingly soluble, dissociable salts.

IV. REAGENTS

The practical value of the solubility method is a function of the availability of reagents. The development and selection of reagents for a given problem is a special study in itself. Only a brief outline can be given here of the manner in which this problem has been approached. The investigation has involved primarily aromatic sulfonic acids (5), although other types of reagents have also been shown to be applicable (3). A few sulfonic acids have already been recommended, in the earlier literature (1, 13), for use in amino acid analysis, flavianic acid (7, 12) being the outstanding example. The general tendency of aromatic sulfonic acids to form sparingly soluble salts with nearly all amino acids, however, has not been recognized until recently. Herein lies a reservoir of reagents potentially useful for the isolation, purification, and determination of amino

TABLE 5

Solubility products of amino acid salts of aromatic sulfonic acids (5)

Approximate molar solubility products determined in *N* HCl at 0°C. Where no value is given, the solubility product of the salt was found to be greater than about 4 × 10^{-2}

AMINO ACIDS	SULFONIC ACIDS											
	p-Chlorobenzene-	*p*-Bromobenzene-	*p*-Iodobenzene-	2,5-Dibromobenzene-†	2,5-Dichlorobenzene-	3,4-Dichlorobenzene-	4-Nitrotoluene-2-	2-Bromotoluene-5-	2,6-Diiodophenol-4-†	*o*-Tosyl-*p*-phenol-	(Flavianic acid) 2,4-Dinitro-1-naphthol-7-	5-Nitronaphthalene-1-†
	×10^{-4}											
l-Alanine												
l-Arginine*				0.2		0.008			0.08	0.06	0.0002	0.02
l-Aspartic acid												
l-Cysteine	100	50	20					20		9		
l-Cystine*										0.06	0.01	
l-Glutamic acid												
Glycine							280				25	5
l-Histidine*				0.2	10	0.002		2	0.002	0.01	0.05	0.6
l-Hydroxyproline									85			9
l-Isoleucine										16		
l-Leucine	330	120	28	57	100	26		9	17	0.3	3	130
l-Lysine*										0.1	0.01	0.2
dl-Methionine	100	100	16		50	16				0.8		
dl-Phenylalanine	120	35	4	0.3	4‡	5	20	10‡	2	0.1	1‡	‡
l-Proline									68			
dl-Serine												
l-Tyrosine	51	190		17	82		28		8	2	0.03	
l-Valine										9		

* The arginine, histidine, lysine, and cystine salts contain 2 moles of sulfonic acid per mole of amino acid. For these ternary salts, $K = [\text{sulfonic acid}]^2 \times [\text{amino acid}]$. Therefore, a comparison of the solubility product of a ternary salt with that of a binary salt is not a direct measure of their relative solubilities. For example, the *l*-arginine and *dl*-phenylalanine salts of 2,5-dibromobenzenesulfonic acid have about the same solubility products. A saturated solution of the ternary arginine salt, however, contains about 0.02 mole of arginine per liter, whereas a saturated solution of the binary phenylalanine salt contains only 0.005 mole of phenylalanine per liter.

† The salts of *l*-cysteine, *l*-cystine, and *dl*-methionine were not investigated in these cases.

‡ *l*-Phenylalanine salts.

acids. In order to choose a reagent for application to any of these problems it is necessary to know first the solubility of the salts which the reagent may form with the major constituents of protein hydrolysates. The type of data which has been gathered for this purpose is illustrated in table 5. Only a few examples of the many reagents investigated are included in this list. For leucine, 2-bromotoluene-5-sulfonic acid can be seen to possess the desired degree of specificity for most present analytical applications; for phenylalanine, 2,5-dibromo- and 2,5-dichloro-benzenesulfonic acids; for glycine, 5-nitronaphthalene-1-sulfonic acid. From data of this type, reagents for additional members of the amino acid series have been developed. Sulfonic acids also may be utilized for the isolation of amino acids and peptides from protein hydrolysates on a preparatory scale. One example is the preparation of *l*-serine and *l*-alanine from silk fibroin (10) with the aid of *p*-hydroxyazobenzene-*p*′-sulfonic acid and azobenzene-*p*-sulfonic acid.

V. CONCLUSION

In the beginning of this discussion it was mentioned that one of the fundamental problems of protein chemistry is the establishment of the complete amino acid composition of the proteins. It may be asked, therefore, to what extent the method outlined in this review may aid in the future accomplishment of this task. It should be emphasized that reagents remain the limiting factor in this type of analytical approach. It is true that the reagent requirements have been lowered, that many additional ones have been developed, and that more quantitative knowledge can be gained today than was possible a few years ago. Yet the task of finding satisfactory reagents for *all* of the amino acids must be recognized as a formidable problem. The extension of the method to additional amino acids will involve considerable further work in both the analytical and the reagent fields. At the present date the method can be summarized as a new and accurate means for the determination of a *number* of the constituent parts of the protein molecule and, as such, is serving as a tool in the general approach to a fuller understanding of protein chemistry.

REFERENCES

(1) Ackermann, D.: Z. physiol. Chem. **225**, 46 (1934).
(2) Bergmann, M., Moore, S., and Stein, W. H.: Unpublished data.
(3) Bergmann, M., and Stein, W. H.: J. Biol. Chem. **128**, 217 (1939).
(4) Bergmann, M., and Stein, W. H.: J. Biol. Chem. **129**, 609 (1939).
(5) Doherty, D. G., Stein, W. H., and Bergmann, M.: J. Biol. Chem. **135**, 487 (1940).
(6) Ing, H. R., and Bergmann, M.: J. Biol. Chem. **129**, 603 (1939).
(7) Kossel, A., and Gross, R. E.: Z. physiol. Chem. **135**, 167 (1924).
(8) Rittenberg, D.: Paper presented at the Symposium on Physiochemical Methods in Protein Chemistry, which was held at the 102nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September 8–12, 1941.
(9) Rittenberg, D., and Foster, G. L.: J. Biol. Chem. **133**, 737 (1940).
(10) Stein, W. H., Moore, S., and Bergmann, M.: J. Biol. Chem. **139**, 481 (1941).
(11) Stein, W. H., Niemann, C., and Bergmann, M.: J. Am. Chem. Soc. **60**, 1703 (1938).
(12) Vickery, H. B.: J. Biol. Chem. **132**, 325 (1940).
(13) Zimmermann, W.: Z. physiol. Chem. **188**, 180 (1930).

THE INVESTIGATION OF PROTEINS BY DIELECTRIC MEASUREMENTS[1]

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The dielectric behavior of a solute of long relaxation time in a solvent of shorter relaxation time is discussed. Procedures adapted to the measurement of both the dielectric constant and the conductance of such systems are outlined, and the effect of electrode polarization upon the results is also discussed.

Such experiments yield data concerning (*1*) the dielectric increments at high and at low frequencies and (*2*) the behavior of the solution in the dispersion region. Interpretation of the increment results in terms of dipole moments and of the dispersion results in terms of the shape and size (and hence the hydration) of the molecules are discussed. Most of the dielectric studies upon protein solution reported in the literature are briefly reviewed.

The interactions of protein molecules with similar protein molecules, with other protein molecules, and with other types of molecules and ions are dependent upon the charge and dipole moment of the molecules or ions involved (7, 9). The dipole moments of protein molecules are often very large and play a most important part in the behavior of protein solutions, and in many cases these studies have been carried out in such a manner that information regarding the size and shape of the protein molecules can be obtained.

I. INTRODUCTION

The dielectric constant, ϵ, of a polar solution can be related to the number of dipoles oriented by the external applied field and the dipole moment, μ, of the individual dipoles. Orientation is hindered by the Brownian motion of the molecules and by frictional forces, these latter forces being proportional to the rate of orientation (measured by the frequency, ν, of the applied field) and a constant τ, designated as the "relaxation time," dependent on the viscosity of the solvent and the size and shape of the dipoles. Thus the degree of orientation at unit field strength will decrease in a frequency region where the hindering frictional forces and the orienting forces become of the same order of magnitude.

Figure 1 represents the dielectric behavior of a solution of protein molecules with relaxation times of the order of microseconds, dissolved in low-molecular-weight polar solvent molecules with relaxation times much smaller than this. The figure is divided into five regions, each of which exhibits a different dielectric behavior. In region A the orienting torque acting on both solute and solvent molecules is sufficient to overcome all frictional forces, and we find both being oriented and a constant and high value of ϵ, designated ϵ_0. In region B, however, the frictional forces on protein dipoles with the larger relaxation time can no longer be neglected, the orientation of these dipoles is no longer independent of the frequency, and we find a region of decreasing dielectric constant. In region C this frictional force has overcome completely the orienting force on the protein dipoles, and they then contribute very little to the dielectric constant of the solution, which we designate ϵ_∞. In regions D and E we have similar changes in the orientation of the solvent dipoles, but in the case of studies where the relaxation times of the solvent molecules are smaller than those of the protein dipoles by a factor of 100 or more, which is usually the case with protein solutions,[2] the dielectric behavior in this region does not complicate that observed in regions A, B, and C, and furnishes little or no information regarding the properties of the protein molecules.

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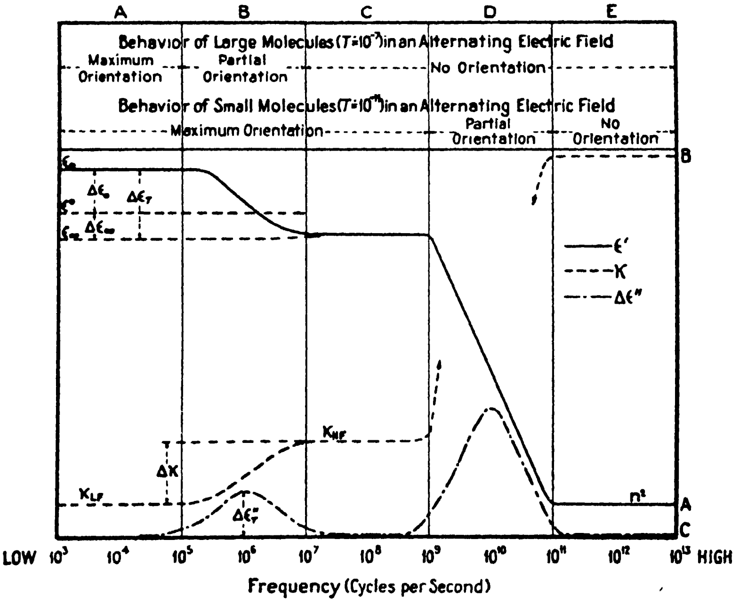

FIG. 1. Schematic diagram of anomalous dispersion of the dielectric constant (ϵ'), the specific conductivity (κ), and the dielectric absorption ($\Delta\epsilon''$) for two widely separated critical frequencies. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40**, 371 (1940)).

II. METHODS OF MEASUREMENT

The measurement of the dielectric properties of protein solutions over an extended frequency range can be carried out by any of several methods which have thus far been devised and employed. They are more or less classifiable as (*1*) bridge methods, (*2*) resonance methods, (*3*) force methods, (*4*) calorimetric methods, and (*5*) various other methods, involving comparisons of phase and magnitude of the voltage across an unknown and a standard.

(*1*) The bridge method involves the comparison of the resistance and capacitance of a cell containing the solution under investigation with some arrangement of standard resistances and capacitances. The main source of difficulty in measurements of this type with solutions of high conductance is in the selection of a suitable resistance standard. Apparatus used for the study of protein solutions has been described by Errera (18), by Daniels, Mathews, and Williams (11), by Oncley (32, 33), and by Ferry and Oncley (21). Apparatus suitable for such measurements has also been described by Cole and Curtis (10) and by Hemingway and McClendon (25). A modification of the bridge described previously (21, 32, 33) is now being used in our laboratory. The standard capacitance is a General Radio Type 722-D precision condenser, and the standard resistance is made up of a General Radio Type 669 compensated slide-wire resistor (1 ohm), of two General Radio Type 668 compensated decade-resistance units (10 and 100 ohms), and a series of 100-ohm resistance units such as are used in the General Radio Type 510 decade-resistance, which are shunted with trimming condensers adjusted to eliminate exactly the effective inductance of the entire resistance combination.[3] The ratio arms are General Radio Type 516-P4 units, of 1000 ohms each. The fourth arm of the bridge is made up of resistance units identical with those used for the standard, and of a series of variable air condensers. The unknown is connected in parallel with the standard arm of the bridge, and measured by substitution methods. By a suitable choice of detector and oscillator equipment, this bridge may be used over a wide frequency range.[4]

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[2] The relaxation time of water at room temperature is of the order of 10^{-11} sec., while that of the amino acids and simpler peptides is 10^{-10} sec.

[3] A brief description of the standard resistance is necessary. Almost any type of resistor has a certain amount of residual capacitance or inductance. The type 668 and 669 units that we have employed have an almost constant residual inductance, and can be represented by a resistance R_a, variable from 0 to 111 ohms, and a series inductance L_a, of about 1.1± 0.05 microhenrys. The type 510 resistance, R_b, shunted with the trimming condenser, C_b, can as a first approximation (i.e., at low frequencies) be considered as a fixed resistance R_b and a fixed series inductance, $-C_bR_b^2$. When these units are connected in series, we have a total series resistance of $R_a + R_b$ and a total series inductance of $L_a - C_bR_b^2$. By a proper adjustment of C_b we can reduce this series inductance to zero at low frequencies. Experimentally we find this to be a perfectly feasible method for obtaining a variable resistor with very low residuals between 1000 and 5,000,000 cycles. The resistance R_b can be set equal to 50, 100, 200, or 300 ohms. Higher resistances cannot easily be used, since the capacitance of the switch and connections is so great that the value $L_a - C_bR_b^2$ becomes negative even without any shunting condenser.

[4] The equipment used in this laboratory consists of the following:

FREQUENCY RANGE	OSCILLATOR	AMPLIFIER	DETECTOR
50 to 8,000	Clough-Brengle Type 79-U audio frequency oscillator	General Radio Type 814A amplifier	General Radio Type 760A sound analyzer
10,000 to 100,000	General Radio Type 684A modulated oscillator	General Radio Type 814A amplifier	Sargent Model 11-UA all-wave receiver
100,000 to 5,000,000	General Radio Type 684A modulated oscillator	None	Sargent Model 11-UA all-wave receiver

(*2*) The resonance method is widely used, especially for the higher range of frequencies (above 10^6 cycles per second). It involves the measurement of the resonance frequency of a circuit which includes a cell containing the solution. It has been widely used by Drude (14) and others. Many modifications have been proposed, the most accurate of which is probably that of Wyman (48). Elliott and Williams (16) have recently described a method applied to protein solutions.

(*3*) The force method involves the measurement of the deflecting force exerted by an applied electric field upon a conducting ellipsoid suspended in the solution under investigation. It was originally described by Fürth (24) and has most recently been applied by Shutt and his coworkers (4, 15, 42). It is most effective at the low frequencies (10^2 to 10^4 cycles per second).

(*4*) The calorimetric method involves the determination of the expansion of the solution as a measure of the heating produced by an applied field of high frequency. It has been used for the study of various solutions by Malsch (29), Debye (13), Martin (30), Schmelzer (40), and others. Its application to protein solutions has been described by Shack (41). It is most effective at the higher frequencies, but is capable of use over most of the usual dispersion range for protein solutions of not too high conductance.

(*5*) Various other methods have been used for the measurement of the conductance and capacitance, or in some cases of the relaxation time directly, of a solution. Most of these methods depend upon the direct comparison of magnitude and phase of the voltage across an unknown and a standard capacitance, often by means of an oscillograph measurement. A recent publication of Wyman and Marcy (52) describes such an apparatus.

III. DIELECTRIC INCREMENT

The dielectric increment of a solution, $\Delta\epsilon$, is defined as the increase in dielectric constant of the solution, ϵ, over that of the solvent under similar conditions, ϵ^0; that is,

$$\Delta\epsilon = \epsilon - \epsilon^0 \tag{1}$$

In the case of most polar solutes in solvents of high dielectric constant, this increment is found to be at least approximately proportional to the concentration (50, 51). In the case of protein solutions, it is convenient to define a quantity $\Delta\epsilon/g$, designated the "increment per gram," where g is the concentration of the protein expressed in grams per liter. This increment per gram is related to the increment per mole, δ, by the equation $\Delta\epsilon/g = \delta/M$, where M is the molecular weight of the solute (51).

Low-frequency increment

The increment measured in the low-frequency region, indicated by A in figure 1, is called the low-frequency increment, and designated $\Delta\epsilon_0$. It is independent of frequency over a wide range of frequencies, if complications due to polarization capacity are eliminated by proper methods of measurement. A linear relationship between the low-frequency increment $\Delta\epsilon_0$ and the concentration has been observed in dilute solutions of a considerable number of proteins. In many cases this linear relationship holds up to quite high concentrations, but in some cases, especially when $\Delta\epsilon_0/g$ is large, the increment per gram decreases somewhat with increasing concentration. In general we may write

$$\Delta\epsilon_0 = E_0 g - F_0 g^2 \tag{2}$$

where E_0 and F_0 represent parameters characteristic of the individual proteins. Terms in higher powers of g may sometime be required, but present indications are that the coefficients for these terms will be small, and negligible except in very concentrated solutions.

High-frequency increment

The increment measured in the high-frequency region, indicated by C in figure 1, is called the high-frequency increment, and designated $\Delta\epsilon_\infty$. The high-frequency increment has been measured for only a few proteins. If we assume that the dielectric constant at high frequencies, ϵ_∞, is due largely to the contribution of solvent molecules (assuming the volume occupied by the protein to have a high-frequency dielectric constant of unity),[5] then we obtain the equation

$$\Delta\epsilon_\infty/g = (\epsilon^0 - 1)v/1000 \tag{3}$$

where v is the volume of water (in cubic centimeters) displaced by 1 g. of anhydrous protein. We can set $v = \bar{v} + w/\rho_0$, where ρ_0 is the density of the solvent, $\bar{v}$ the partial specific volume of the solute (anhydrous), and w the number of grams of water which appear to be associated with each gram of anhydrous protein. If $w = 0$, and $\bar{v} = 0.75$, we obtain a value of $\Delta\epsilon_\infty/g = 0.059$ for water at 25°C. and 0.060 at 0°C. The observed values are of this order of magnitude, but slightly larger. If we assume reasonable values for w (34), the agreement is very good.

Total increment

The total increment is designated $\Delta\epsilon_t$, and is the sum of the low-frequency and high-frequency increments. In cases where proteins are dissolved in mixed solvents, there is sometimes difficulty in exactly defining the composition of the solvent, since one or more of the components may be more strongly absorbed by the protein molecules. In these cases it is not possible to compute accurately the low-frequency and high-frequency increments, but the total increment may still be accurately obtained.

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[5] The value of unity for the high-frequency dielectric constant of the protein is too small, since it neglects entirely the optical polarization of the protein and the polarization of the hydrated water molecules. The value $(\epsilon^0 - 1)$ in equation 3 is, however, probably within 5 or 10 per cent of the proper value for solvents of high dielectric constant.

IV. ELECTRODE POLARIZATION

One of the most serious difficulties involved in measurements by any of these methods is the complication introduced by electrode polarization. This effect is most serious at low frequencies and high conductivities, but decreases in magnitude until it may properly be neglected at high frequencies or for solutions of very low conductivity. It is discussed in some detail by Jones and Christian (26), by Cole and Curtis (10), by Fricke and Curtis (22), by Oncley (32), by Ferry and Oncley (21), and by Dunning and Shutt (15) and must be considered as a possible source of error in all work of this type. Correction for this effect was made very successfully at frequencies higher than 25,000 cycles for salt solutions and protein solutions (32) by plotting capacities against $\nu^{-3/2}$ and determining B empirically as the slope of the resulting straight line. The dielectric increment $\Delta\epsilon$ is thus given by

$$\Delta\epsilon = (C_x - B\nu^{-3/2} - C_x^0)/Q \tag{4}$$

where C_x is the cell capacity at frequency ν with the protein or salt solution, C_x^0 the cell capacity with conductivity water, and Q the cell constant ($dC_x/d\epsilon'$). Below 25,000 cycles, however, the plot against $\nu^{-3/2}$ was often found to deviate from linearity, and it was no longer possible to correct for the polarization capacity effect by this simple procedure. To involve the least additional complication, a method of comparison between solutions of equal conductivities has been employed. If the polarization correction is represented by $B\nu^{-3/2} + f(\nu)$, where $f(\nu)$ takes care of the departure from linearity of the $\nu^{-3/2}$ plot, and if $f(\nu)$ should prove to be dependent only on the conductivity, but not on the nature of the solute, then the difference between the corrections for two solutions of different solutes, but with the same conductivity, should be proportional to $\nu^{-3/2}$. The parameter B will in general not be the same for the two solutions, but the difference, ΔB, can be determined from the plot of $(C_x - C_x')$ against $\nu^{-3/2}$, where C_x' is the capacity of the cell when filled with a potassium chloride solution of the same conductivity, and the values of $\Delta\epsilon$ obtained as

$$\Delta\epsilon = (C_x - C_x' - \Delta B\nu^{-3/2})/Q \tag{5}$$

This method has been tested by a series of measurements comparing solutions of potassium chloride, ammonium sulfate, glycine (with potassium chloride added to adjust the conductivity), and lactoglobulin in glycine, and proved to be satisfactory for frequencies above 10,000 cycles (21).

V. CALCULATION OF DIPOLE MOMENTS

The interpretation of dielectric increments obtained in polar solvents in terms of the dipole moments of the solute molecules has been discussed by numerous workers (23, 28, 31, 37, 43–45, 47, 50, 51). In the case of protein measurements, where both the high-frequency and the low-frequency increments can be obtained, we can eliminate some of the approximations which are often introduced. This is especially important when we are dealing with proteins with small values of the low-frequency increment per gram. The relation between dipole moment and dielectric increment may be expressed by the equation

$$\mu^2 = [9000kT/4\pi Nb]\,[M(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)] \tag{6}$$

where N is Avogadro's number, k is Boltzmann's constant, T is the absolute temperature, and μ is the dipole moment of a single molecule in solution. This moment is not necessarily equal to the moment of a single molecule in the gas phase, but is in general somewhat greater (28).

The parameter b may be evaluated in several different ways:

(*1*) Direct application of the Debye theory to polar media (32) yields a value $b = (\epsilon_0 + 2)(\epsilon_\infty + 2)/3$, which may be approximated by the equation $b = (\epsilon^0 + 2)^2/3$ for solutions with sufficiently small increments.

(*2*) Studies by Wyman and others indicate that $b = (\epsilon - a)/p$ in pure liquids and $b = (\epsilon - \epsilon^0 - a)/p$ in binary mixtures, where p is the volume polarization and a another parameter. Wyman (50) suggests the values $b = 8.5$ and $a = -1$ as best representing the bulk of the available data.

(*3*) We may evaluate b by assuming values of $(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)$ and μ for the glycine molecule. Various measurements[6] indicate that μ is close to 15, and we will choose this value. The total increment per gram may be taken as 0.36.[7]

(*4*) Onsager (37) has computed b as a function of an internal refractive index, n, giving the result $b = (n^2 + 2)^2/2 = 4.5\,[1 + (n^2 - 1)/3]^2$.

(*5*) Kirkwood (28) has presented a method for computing b in terms of the hindered relative rotation of neighboring molecules. He gives the equation

$$b = 4.5\left[1 + \frac{N}{v}\int^{v^0} \cos\gamma e^{-W/kT}\,d\omega\,dv\right] = 4.5(1 + \beta)$$

where

$$\int^{v^0} e^{-W/kT}\,d\omega\,dv = 1$$

Here γ is the angle between the dipole moments of an arbitrary pair of molecules, and W is the potential between an arbitrary pair of molecules. The integration extends over all relative orientations ($d\omega$) and positions (dv) within a sphere of volume v_0 which includes the molecule and the first few shells of neighboring molecules.

For the calculation of dipole moments it is convenient to modify equation 6 to become:

$$\mu = \alpha\sqrt{M(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)} = \alpha\sqrt{M(\Delta\epsilon_t/g)}$$

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[6] Kirkwood (27) estimates the moment as 15.0 Debye units, on the basis of studies of the solvent action of neutral salts on glycine at low dielectric constants by Cohn (5), and Scatchard and Prentiss (39) estimate the moment as 14.8 Debye units, on the basis of freezing-point measurements. A value near 15 is obtained by consideration of the distance separating the positive and negative charges in the glycine molecule.

[7] $(\Delta\epsilon_0/g)$ from Wyman and McMeekin (53) and $(\Delta\epsilon_\infty/g)$ calculated from equation 3, taking 0.582 for the partial specific volume of glycine (6).

Here a new parameter

$$\alpha = \sqrt{9000kT/(4\pi Nb)} = 0.403 \times 10^{-18}\sqrt{T/b}$$

has been introduced, and values of both b and this quantity at 0°, 20°, and 25°C. are tabulated in table 1. Values of n calculated from Onsager's equation and of β calculated from Kirkwood's equation are also given for the various values of b.

The assumption of $b = 5.8$, as obtained by method (*3*) using 15 for the dipole moment of glycine, appears to be the most reasonable choice we can make at the present time, and the values for α calculated from this b value are those used in the calculation of all protein dipole moments. It would seem unlikely that these values should vary more than perhaps ±15–25 per cent from the true ones.

VI. DISPERSION STUDIES

The study of the region B, figure 1, in which dielectric dispersion occurs is capable of giving us information dependent upon the size and shape of the molecules, and gives us one of the most powerful methods for the accurate measurement of these quantities that is at present available.

TABLE 1

Various evaluations of b and α for equation 7 and the corresponding values for the dipole moment of glycine

	b	$\alpha \times 10^{18}$			DIPOLE MOMENT OF GLYCINE	ONSAGER'S REFRACTIVE INDEX, n	KIRKWOOD'S PARAMETER, β
		0°C.	20°C.	25°C.			
Debye	2160	0.136	0.146	0.149	0.77	8.1	479
Wyman	8.5	2.28	2.36	2.38	12.3	1.457	0.89
Empirical	5.8	2.76	2.86	2.89	15.0	1.182	0.29
Onsager or Kirkwood*	4.5	3.13	3.24	3.28	17.0	1.000	0.0

* Minimum values for b.

Dispersion of dielectric constant

The quantitative behavior of the dielectric constant in this dispersion region is somewhat complex. Debye (12) has treated the simplest case involving a single relaxation time, and has obtained the equations

$$\epsilon' = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)/(1 + \nu^2/\nu_c^2) \qquad (8)$$

Here ϵ' is the usual dielectric constant of the solution (often called the "real" dielectric constant), ϵ_0 and ϵ_∞ are the dielectric constants observed in regions A and C, respectively, ν is the frequency, usually expressed in cycles per second, or megacycles per second, and ν_c is the "critical frequency," defined as $1/(2\pi\tau)$, where τ is the relaxation time, which is the time required for $1/e$ of the molecules to become randomly distributed if they were completely oriented by a field, and then released at $t = 0$.

This simple theory, involving a single relaxation time and critical frequency, can be extended to include the case of several relaxation times, giving

$$\epsilon' = \epsilon_\infty + \Delta\epsilon_1/(1 + \nu^2/\nu_1^2) + \Delta\epsilon_2/(1 + \nu^2/\nu_2^2) + \cdots \qquad (9)$$

where $\Delta\epsilon_1$, $\Delta\epsilon_2$, etc., represent the total dielectric increment associated with the critical frequencies ν_1, ν_2, etc. The critical frequencies are defined as before; that is, $\nu_i = 1/(2\pi\tau_i)$. An ellipsoidal molecule would in general have three relaxation times, but if we restrict ourselves to ellipsoids of revolution, then we have only two relaxation times, τ_a and τ_b, and hence two critical frequencies, ν_a and ν_b. Equations of Perrin (39) give τ_a and τ_b in terms of the ratio of axes of the ellipsoid, a/b, and the relaxation time τ_0 of a sphere of the same volume, and we can thus express ν_a and ν_b in terms of a/b and ν_0. If $\Delta\epsilon_t$ be taken as the sum of $\Delta\epsilon_a$ and $\Delta\epsilon_b$, then equation 7 can be expressed in terms of a/b, ν_0, $\Delta\epsilon_t$, and $\Delta\epsilon_a/\Delta\epsilon_b$, and we can construct series of curves of $(\epsilon' - \epsilon_\infty)/\Delta\epsilon_t$ against ν/ν_0 for various values of asymmetry, a/b, and increment ratio, $\Delta\epsilon_a/\Delta\epsilon_b$. Such curves are illustrated in figures 2 and 3. By comparison of the experimental values obtained in region B (figure 1) with theoretical values calculated in this manner, we can evaluate a/b, $\Delta\epsilon_a/\Delta\epsilon_b$, and ν_0. The values of ν_0 (or τ_0) are directly related to the molecular volumes, V, of the particles:

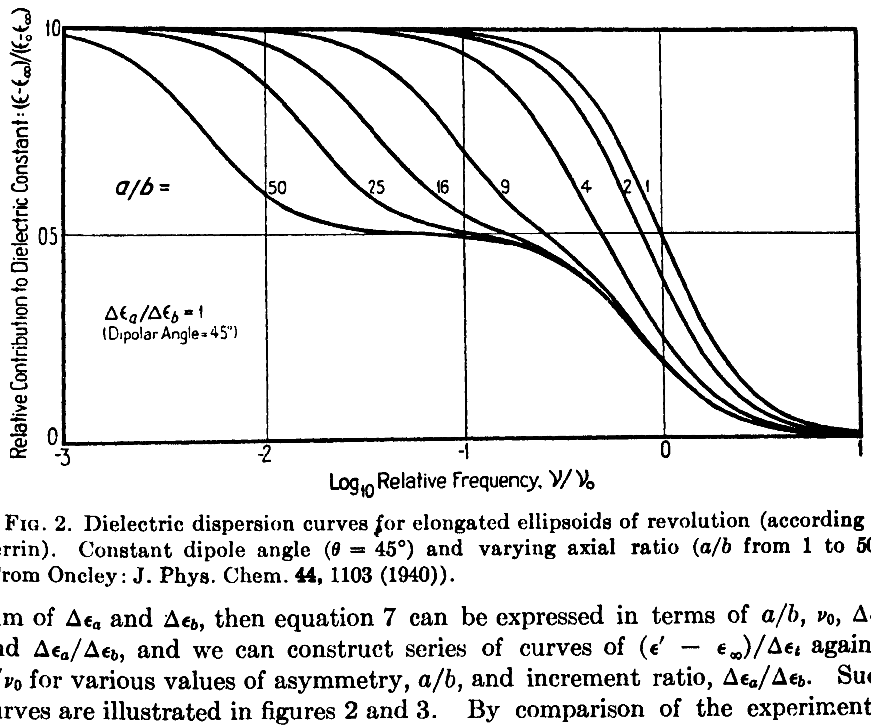

FIG. 2. Dielectric dispersion curves for elongated ellipsoids of revolution (according to Perrin). Constant dipole angle (θ = 45°) and varying axial ratio (a/b from 1 to 50). (From Oncley: J. Phys. Chem. **44**, 1103 (1940)).

$$\nu_0 = kT/(8\pi^2ab^2\eta) = RT/(6\pi V\eta) = 1/(2\pi\tau_0) \qquad (10)$$

or

$$V = RT/(6\pi\eta\nu_0) = RT\tau_0/(3\eta) \qquad (11)$$

For water at 25°C., this becomes

$$V = 147{,}000/(\nu_0)_{25,w} = 9250(\tau_0)_{25,w} \times 10^8 \qquad (11a)$$

where ν_0 is expressed in megacycles and τ_0 in seconds. The relaxation times are usually reduced to the value which would be obtained at 25°C. in water, using the equation

$$\tau_{25,w} = \tau T/(298 \cdot \eta/\eta_{25,w}) = T/(2\pi\nu_c \cdot 298 \cdot \eta/\eta_{25,w}) \qquad (11b)$$

The increment ratio, $\Delta\epsilon_a/\Delta\epsilon_b$, may also be expressed as a "dipole angle," θ, defined as the angle between the geometric axis a of the ellipsoid and the electric moment vector. Since the electric moment μ will be the vector sum of the two moments μ_a and μ_b, and since the moments are proportional to the square roots of the increments, we have

$$\tan \theta = \mu_b/\mu_a = \sqrt{\Delta\epsilon_b/\Delta\epsilon_a} \qquad (12)$$

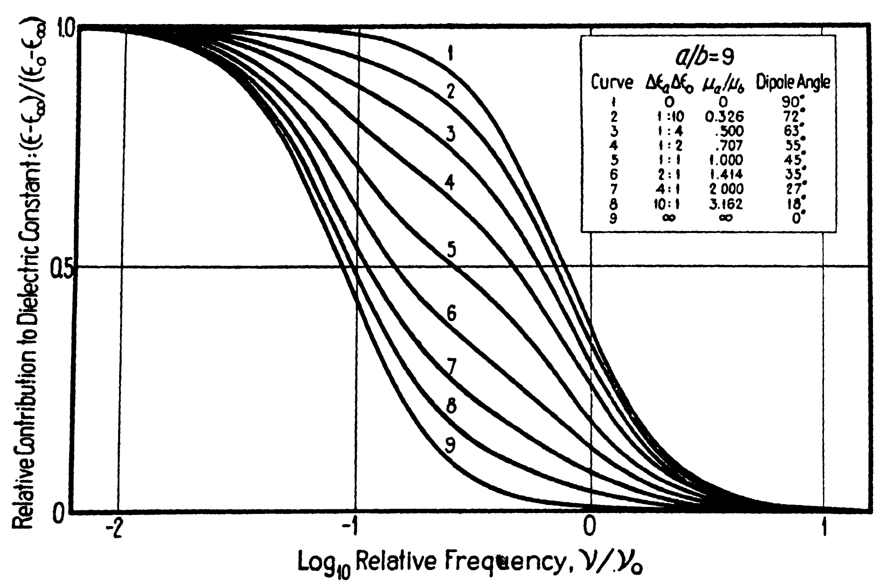

FIG. 3. Dielectric dispersion curves for elongated ellipsoids of revolution (according to Perrin). Constant axial ratio ($a/b = 9$) and varying dipole angle (θ from 0° to 90°). (From Oncley: J. Phys. Chem. **44**, 1103 (1940)).

Dispersion of conductance

Measurements of the specific conductance,[8] κ, of a protein solution can also be used to evaluate the total dielectric increment and relaxation times of mole-

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[8] The specific conductance of a solution, κ, is determined from the measured parallel conductance, G, by the equation $\kappa = 0.0885G/Q = kG$, where k is the usual conductance cell constant and Q is the change in cell capacitance (in $\mu\mu$fds) caused by a unit change in the dielectric constant ($Q = dC/d\epsilon'$). Here G is expressed in μmhos and κ in μmhos per centimeter.

We may also express the dielectric losses in these solutions in terms of the imaginary part of the complex dielectric constant, or by the phase angle. The complex dielectric constant, ϵ, is composed of a real part, ϵ', usually simply called the dielectric constant, and an imaginary part, ϵ''. We have $\epsilon = \epsilon' - i\epsilon''$, where $i = \sqrt{-1}$. The phase angle, δ, is cules. At low frequencies (region A, figure 1) this conductance will have the value κ_0, which increases in region B to the value κ_∞ in region C. We thus have the equations

$$\kappa = \kappa_\infty - (\kappa_\infty - \kappa_0)/(1 + \nu^2/\nu_c^2) = \kappa_0 + (\kappa_\infty - \kappa_0)(\nu/\nu_c)^2/(1 + \nu^2/\nu_c^2) \quad (13)$$

and

$$\begin{aligned} \kappa &= \kappa_\infty - \Delta\kappa_1/(1 + \nu^2/\nu_1^2) - \Delta\kappa_2/(1 + \nu^2/\nu_2^2) - \cdots \\ &= \kappa_0 + \Delta\kappa_1(\nu/\nu_1)^2/(1 + \nu^2/\nu_1^2) + \Delta\kappa_2(\nu/\nu_2)^2/(1 + \nu^2/\nu_2^2) \cdots \quad (14) \end{aligned}$$

TABLE 2

*Comparison of results obtained on several protein molecules studied by both bridge and calorimetric methods**

PROTEIN	METHOD	CRITICAL FREQUENCY ν_c	DIELECTRIC INCREMENT $\Delta\epsilon_i/g$	CONDUCTANCE INCREMENT $\Delta\kappa_i/g$
Egg albumin (8–9 per cent concentration), 1st relaxation	Bridge	0.88	0.092	(0.045)
	Calorimetric	0.80	(0.102)	0.046
	Best values	0.86	0.094	0.045
Egg albumin (8–9 per cent concentration), 2nd relaxation	Bridge	3.6	0.071	(0.142)
	Calorimetric	3.3	(0.062)	0.114
	Best values	3.4	0.063	0.119
Egg albumin (8–9 per cent concentration), total increments	Bridge		0.163	(0.187)
	Calorimetric		(0.165)	0.154
	Best values		0.157	0.164
Carboxyhemoglobin (horse)	Bridge	1.9	0.44	(0.46)
	Calorimetric	2.0	(0.40)	0.44
	Best values	1.9	0.42	0.44
Lactoglobulin (0.1 per cent in water)	Bridge	2.4	1.5	(2.0)
	Calorimetric	2.2	(1.4)	1.7
	Best values	2.4	1.5	2.0

* Taken in part from Oncley, Ferry, and Shack (36).

to correspond with equations 8 and 9 for the dielectric constant. Here $\Delta\kappa_i$ and ν_i represent the conductance increment and critical frequency of the i^{th} dispersion region. The relation

$$\Delta\kappa_i = \Delta\epsilon_i\nu_i/1.80 \quad (15)$$

---

defined by the equation $\tan\delta = \epsilon''/\epsilon'$. When dealing with solutions having D.C. conductance, κ_0, we must not include this part of the loss when computing the imaginary dielectric constant. The value as corrected for this D.C. conductance has been designated $\Delta\epsilon''$, and called the dielectric absorption (36, 41). It is related to the conductance by the equation $\Delta\epsilon'' = 1.80\ (\kappa - \kappa_0)/\nu$. Here the specific conductance κ (and κ_0) is expressed in μmhos per centimeter, and the frequency ν in megacycles per second.

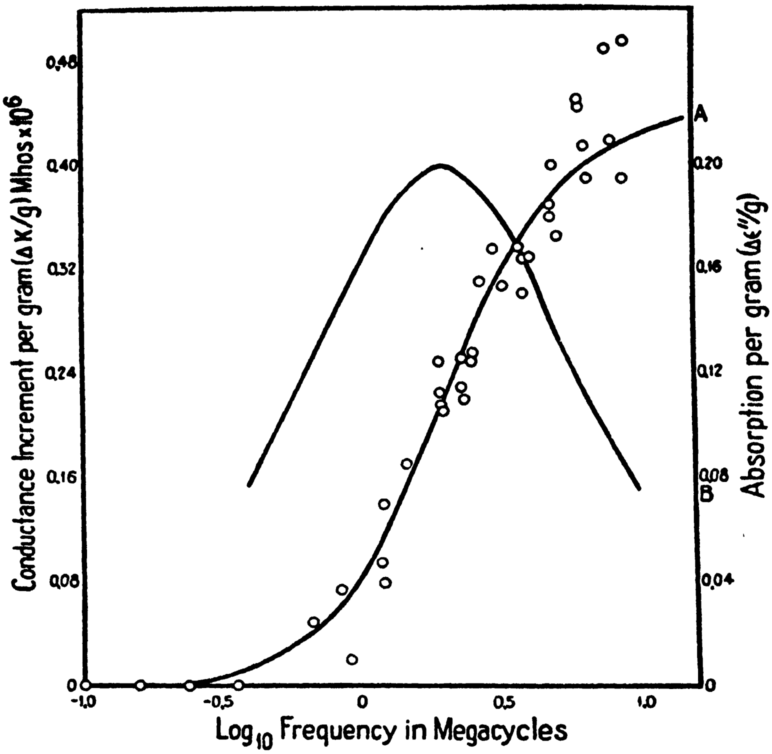

Fig. 4. Dielectric absorption of carboxyhemoglobin solutions: o, experimental measurements of $(\kappa - \kappa_0)/g$; curve A, $(\kappa - \kappa_0)/g$, and curve B, $\Delta\epsilon''/g$. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40,** 371 (1940)).

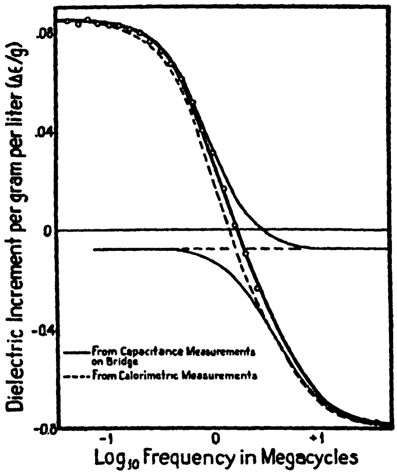

Fig. 5. Dispersion of the dielectric constant of egg albumin, showing resolution into two Debye curves: o, experimental measurements of $\Delta\epsilon/g$; heavy curve from bridge measurements, and broken curve from calorimetric measurements. (From Oncley, Ferry, and Shack: Ann. N. Y. Acad. Sci. **40,** 371 (1940)).

TABLE 3

Dielectric increments and dipole moments of various protein molecules

PROTEIN	SOLVENT	REFERENCE	TEMPERATURE °C.	E_0	F_0	$-E_\infty$	E_t	M	μ
Horse carboxyhemoglobin	Water	(18, 32, 41)	25	0.33	0	0.09	0.42	67,000	480
Pig carboxyhemoglobin	Water	(3)	20				0.3	(67,000)	410
Myoglobin	Water	(52)	25	0.15		(0.06)	0.21	17,000	170
Insulin	80% aqueous propylene glycol	(8)	25				0.38	40,000	360
Insulin	90% aqueous propylene glycol	(8)	25				0.29	40,000	310
Insulin	100% propylene glycol	(8)	25				0.26	40,000	300
Lactoglobulin	$M/2 + M/4$ glycine	(19, 21)	25	1.51	0.025	(0.07)	1.58	40,000	730
Lactoglobulin	$M/2 + M/4$ glycine	(21)	0	1.84	0.047	(0.08)	1.92	40,000	770
Egg albumin	Water	(35, 36, 41)	25	0.10	0	0.07	0.17	44,000	250
Horse serum albumin (carbohydrate-free)	Water	(20, 33)	25	0.17	0	0.07	0.24	70,000	380
Horse serum pseudoglobulin-γ	Water	(20, 33)	25	1.08	0.017	(0.06)	1.14	142,000	1100
Horse serum pseudoglobulin-γ	Water	(33)	0	1.26	0.023	(0.06)	1.32	142,000	1300
Edestin	2 M glycine	(33)	25	0.7		(0.1)	0.8	310,000	1400
Gliadin	56% aqueous ethanol	(2, 17)	25				0.10	42,000	190
Secalin	54% aqueous ethanol	(1)	25				1.0	24,000	440
Zein	72% aqueous ethanol	(16, 46, 49)	25				0.45	40,000	380

exists between the conductance increment, the critical frequency, and the dielectric increment of any dispersion region when conductances are expressed in μmhos per centimeter and critical frequencies in megacycles. Measurements

TABLE 4

Critical frequencies, relaxation times, and geometrical asymmetries of various protein molecules

PROTEIN	SOLVENT	REFERENCE	TEMPERATURE °C.	$\frac{\eta}{\eta_{25,w}}$	$\nu_{\text{obsd.}}$	$\frac{\Delta\epsilon_1}{\Delta\epsilon_2}$	$\tau_{25,w} \times 10^8$	$(\tau_0)_{25,w} \times 10^8$	a/b	$\Theta(°)$
Horse carboxyhemoglobin	Water	(18, 32, 41)	25	1.00	1.9		8.4	(6.6)	(1.6)	
Pig carboxyhemoglobin	Water	(3)	20	1.12	1.1		13.0			
Myoglobin	Water	(52)	25	1.00	5.5		2.9			
Insulin	80% aqueous propylene glycol	(8)	25	17.0	0.59		1.6			
Insulin	90% aqueous propylene glycol	(8)	25	28.0	0.31		1.8			
Insulin	100% propylene glycol	(8)	25	48.0	0.19		1.8			
Lactoglobulin	*M*/4 glycine	(19, 21)	25	1.04	1.0; 3.0	0.25	15; 5.1	4.3	4	63
Lactoglobulin	*M*/2 glycine	(19, 21)	25	1.08	0.9; 2.6	0.25	16; 5.7	4.8	4	63
Lactoglobulin	*M*/2 glycine	(21)	0	2.12	0.44; 1.25	0.25	16; 5.5	4.6	4	63
Egg albumin	Water	(35, 36, 41)	25	1.00	0.86; 3.4	1.5	18; 4.7	3.7	5	40
Horse serum albumin (carbohydrate-free)	Water	(20, 33)	25	1.00	0.44; 2.1	1.0	36; 7.5	6.0	6	45
Horse serum pseudoglobulin-γ	Water	(20, 33)	25	1.00	0.064; 0.57	1.0	250; 28	22.0	9	45
Edestin	2 *M* glycine	(33)	25	1.33	0.050; 0.44	1.0	240; 27	21.0	9	45
Gliadin	56% aqueous ethanol	(2, 17)	25	2.58	0.23; 1.6	1.6	27; 3.8	3.1	8	38
Secalin	54% aqueous ethanol	(1)	25	2.59	0.21; 2.3	1.5	29; 2.7	2.1	10	40
Zein	72% aqueous ethanol	(16, 46, 49)	25	2.22	0.30; 1.7	2.0	24; 4.2	3.3	7	35
Zein		(16)	0.8	5.10		2.0	20; 3.5	2.8		

of this type on a solution yielding a single relaxation time are shown in figure 4. The results of a comparison of conductance increments, dielectric increments, and critical frequencies by independent bridge and calorimetric methods are recorded in table 2. The mean values in this table are weighted, more weight being given to the low-frequency bridge data and to the high-frequency calorimetric data. Values enclosed in parentheses are calculated from equation 15. Figure 5 also compares the data obtained by these two methods in the case of egg albumin.

VII. RESULTS

A survey of the dielectric constant literature for protein solutions reveals few data of significance before those of Wyman (49). Before this time most workers had made measurements at such high frequencies that the results obtained fell in region C (figure 1), where the contribution of the protein molecules to the dielectric constant was very small, and even these measurements were very uncertain. The development of new techniques of measurement since 1928 have made observations in regions A and B much more reliable. The results which we will consider are confined almost entirely to these more recent investigations.

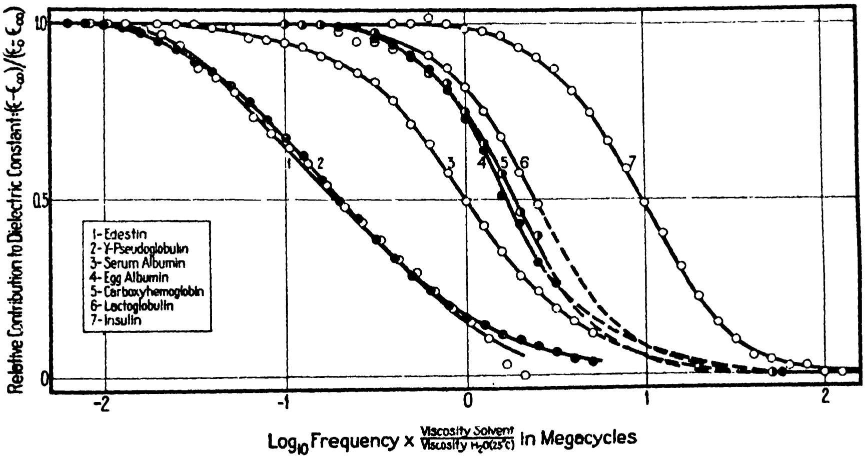

FIG. 6. Dielectric dispersion curves of various proteins. 1, edestin; 2, horse γ-pseudoglobulin; 3, horse serum albumin; 4, egg albumin; 5, horse carboxyhemoglobin; 6, lactoglobulin; 7, insulin.

Dielectric increments

The dielectric increments and dipole moments of a number of protein molecules are recorded in table 3. The observed low-frequency increments vary from about 0.1 to over 1.8, a variation somewhat greater than has been observed in studies upon amino acids and peptides (33, 51). Dipole moments of from less than 200 to about 1400 Debye units have been computed from these results, using equation 7 with *b* equal to 5.8. These values of dipole moment seem very large when compared with those of low-molecular-weight substances, but this is due largely to the high molecular weights of the proteins. A comparison of

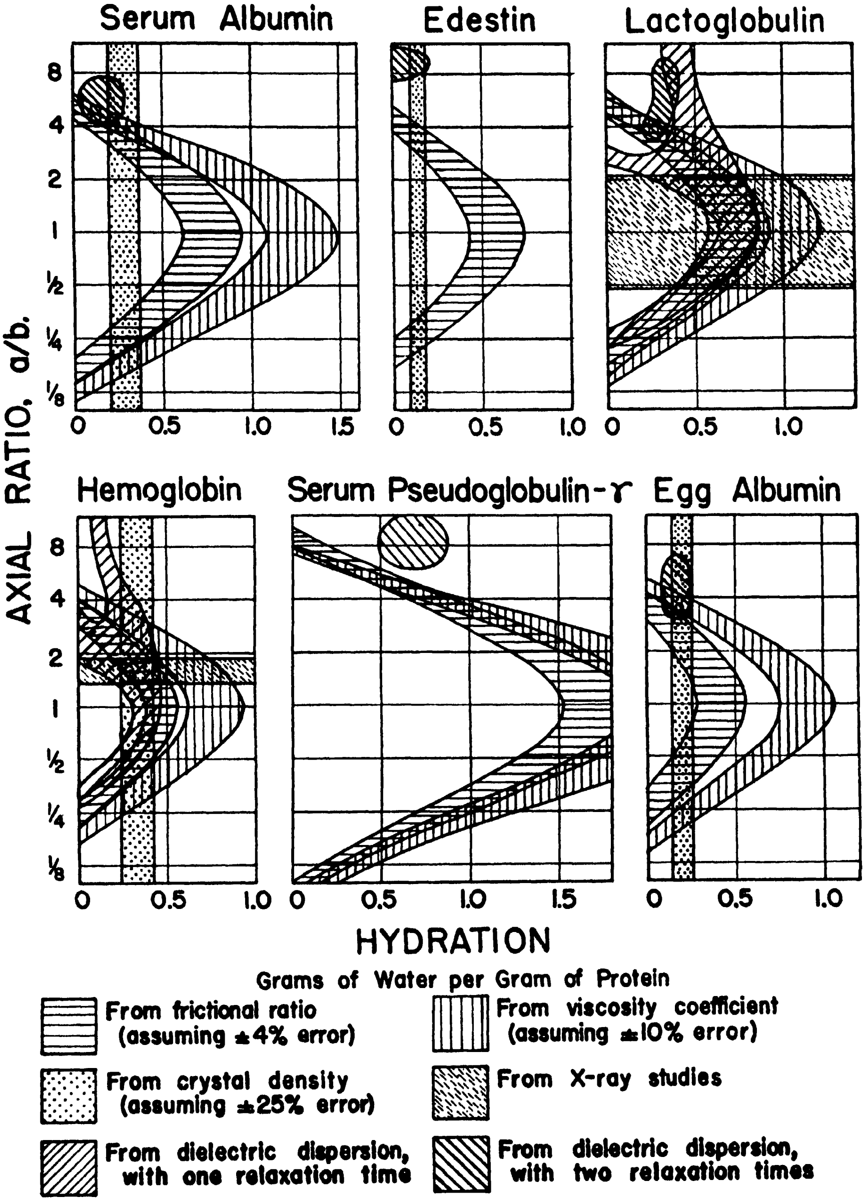

FIG. 7. Asymmetry and hydration of various protein molecules. (Taken in part from Oncley: Ann. N. Y. Acad. Sci. 41, 121 (1941)).

the electrical symmetry of molecules of widely differing size can be made by calculating the diameter, *R*, which the molecule would have if it were spherical and unhydrated, and then computing the number of unit charges, z, which must be located at each end of a dipole of this length in order to give the observed dipole moment.[9] Values obtained in this way are all much smaller than half the total anionic and cationic charges, which is roughly the maximum possible value, $z_{\max.}$. The ratio $z/z_{\max.}$ obtained for serum albumin is about 0.02, for hemoglobin 0.03, and for edestin 0.01. These molecules would accordingly have moments many times those reported here if they were very unsymmetrical electrically, and these measurements can be taken as proof of a fairly high degree of electrical symmetry.

Relaxation times

The observed critical frequencies and relaxation times of most of the proteins which have been studied are recorded in table 4. These values have been obtained from dispersion curves, such as illustrated in figure 6. In the cases where two critical frequencies have been observed, the data have been interpreted in terms of the geometrical asymmetry a/b, the dipole angle θ, and the relaxation time τ_0 of a sphere of equal volume. These values can be expressed in terms of the asymmetry a/b and the hydration of the protein molecules, w, and these quantities can be compared with those obtained by diffusion, ultracentrifuge, x-ray, and other methods. Figure 7 illustrates this comparison, the results being recorded as areas which represent probable combinations of asymmetry and hydration. The agreement is fairly good in most cases, and all of the molecules recorded here would appear to be more or less elongated ellipsoids with hydration values from 0.2 to perhaps 0.6.

When these results are compared with similar studies on other macromolecules, they appear to indicate a characteristic degree of rigidity in protein structure which is not found in most materials.

REFERENCES

(1) Andrews, A. C.: J. Am. Chem. Soc. **62,** 942 (1940); see also Ph.D. dissertation, University of Wisconsin, 1938.
(2) Arrhenius, S.: J. Chem. Phys. **5,** 63 (1937).
(3) Arrhenius, S.: Physik. Z. **39,** 559 (1938); Nova Acta Regiae Soc. Sci. Upsaliensis [4] **12,** No. 5 (1941).
(4) Carr, W., and Shutt, W. J.: Trans. Faraday Soc. **35,** 579 (1939).
(5) Cohn, E. J.: Naturwissenschaften **20,** 44 (1932).
(6) Cohn, E. J.: Ann. Rev. Biochem. **4,** 122 (1935).
(7) Cohn, E. J.: Chem. Rev. **19,** 241 (1936); Harvey Lectures, p. 124 (1938–39).
(8) Cohn, E. J., Ferry, J. D., Livingood, J. J., and Blanchard, M. H.: Science **90,** 183 (1939).
(9) Cohn, E. J., McMeekin, T. L., Ferry, J. D., and Blanchard, M. H.: J. Phys. Chem. **43,** 169 (1939).

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[9] We have used the equation

$$z = \mu/(eR) = 0.41\ [(\Delta\epsilon_t/g)^{1/2}M^{1/6}/\bar{v}^{1/3}] = 0.41\ [\delta_t^{1/2}/V^{1/3}]$$

where M is the molecular weight, $(\Delta\epsilon_t/g)$ the total dielectric increment per gram per liter, and $\bar{v}$ the partial specific volume of the protein, and δ_t and V are the corresponding molal total increment and volume.

(10) Cole, K. S., and Curtis, H. J.: Rev. Sci. Instruments **8**, 333 (1937).

(11) Daniels, F., Mathews, J. H., and Williams, J. W.: *Experimental Physical Chemistry*, 3rd edition, p. 378. McGraw-Hill Book Company, Inc., New York (1941).

(12) Debye, P.: *Polar Molecules*, Chap. V. The Chemical Catalog Company, Inc., New York (1929).

(13) Debye, P.: Trans. Faraday Soc. **30**, 679 (1934).

(14) Drude, P.: Z. physik. Chem. **23**, 267 (1897); **40**, 635 (1902).

(15) Dunning, W. J., and Shutt, W. J.: Trans. Faraday Soc. **34**, 467, 479 (1938).

(16) Elliott, M. A., and Williams, J. W.: J. Am. Chem. Soc. **61**, 718 (1939).

(17) Entrikin, P. P.: J. Am. Chem. Soc. **63**, 2127 (1941).

(18) Errera, J.: J. chim. phys. **29**, 577 (1932).

(19) Ferry, J. D., Cohn, E. J., Oncley, J. L., and Blanchard, M. H.: J. Biol. Chem. **128**, Proc. xxviii (1939).

(20) Ferry, J. D., and Oncley, J. L.: J. Am. Chem. Soc. **60**, 1123 (1938).

(21) Ferry, J. D., and Oncley, J. L.: J. Am. Chem. Soc. **63**, 272 (1941).

(22) Fricke, H., and Curtis, H. J.: J. Phys. Chem. **40**, 715 (1936); **41**, 729 (1937).

(23) Fuoss, R. M.: J. Am. Chem. Soc. **56**, 1031 (1934); Physik. Z. **35**, 59 (1934).

(24) Fürth, R.: Z. Physik. **22**, 98 (1924); Handbuch der biologischen Arbeitsmethoden IIIB, 835 (1929).

(25) Hemingway, A., and McClendon, J. F.: Physics **2**, 396 (1932).

(26) Jones, G., and Christian, S. M.: J. Am. Chem. Soc. **57**, 272 (1935).

(27) Kirkwood, J. G.: J. Chem. Phys. **2**, 351 (1934).

(28) Kirkwood, J. G.: J. Chem. Phys. **7**, 911 (1939).

(29) Malsch, J.: Physik. Z. **33**, 19 (1932); Ann. Physik **12**, 865 (1932).

(30) Martin, G.: Physik. Z. **37**, 665 (1936).

(31) Müller, F. H.: Physik. Z. **34**, 688 (1933); Trans. Faraday Soc. **30**, 729 (1934).

(32) Oncley, J. L.: J. Am. Chem. Soc. **60**, 1115 (1938).

(33) Oncley, J. L. J. Phys. Chem. **44**, 1103 (1940).

(34) Oncley, J. L. Ann. N. Y. Acad. Sci. **41**, 121 (1941).

(35) Oncley, J. L.: Unpublished data.

(36) Oncley, J. L., Ferry, J. D., and Shack, J.: Ann. New York Acad. Sci. **40**, 371 (1940).

(37) Onsager, L.: J. Am. Chem. Soc. **58**, 1486 (1936).

(38) Perrin, F.: J. phys. radium **5**, 497 (1934).

(39) Scatchard, G., and Prentiss, S. S.: J. Am. Chem. Soc. **56**, 2314 (1934).

(40) Schmelzer, C.: Physik. Z. **37**, 162 (1936); Ann. Physik **28**, 35 (1937).

(41) Shack, J.: Ph.D. dissertation, "Dielectric Absorption of Protein Solutions," Harvard University, 1939.

(42) Shutt, W. J.: Trans. Faraday Soc. **30**, 893 (1934).

(43) Van Vleck, J. H.: J. Chem. Phys. **5**, 320, 556 (1937).

(44) Williams, J. W.: Trans. Faraday Soc. **30**, 723 (1934); J. Franklin Inst. **219**, 211 (1935).

(45) Williams, J. W., and Oncley, J. L.: Physics **3**, 318 (1932).

(46) Williams, J. W., and Watson, C. A.: Cold Spring Harbor Symposia Quant. Biol. **6**, 208 (1938).

(47) Wilson, J. N.: Chem. Rev. **25**, 377 (1939).

(48) Wyman, J., Jr.: Phys. Rev. **35**, 623 (1930).

(49) Wyman, J., Jr.: J. Biol. Chem. **90**, 443 (1931).

(50) Wyman, J., Jr.: J. Am. Chem. Soc. **58**, 1482 (1936).

(51) Wyman, J., Jr.: Chem. Rev. **19**, 213 (1936).

(52) Wyman, J., Jr., and Marcy, H. O.: J. Am. Chem. Soc. **63**, 3388 (1941); **64**, 638 (1942).

(53) Wyman, J., Jr., and McMeekin, T. L.: J. Am. Chem. Soc. **55**, 908 (1933).

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